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THE INFLUENCE OF SOIL AND AIR TEMPERATURES ON CROPPING OF GLASSHOUSE TOMATOES

By A. CALVERT
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THE belief that an increase in the temperature of glasshouse soils or other rooting media enhances plant response, is still widely held. Many attempts have been made to increase the cropping of tomatoes by growing the plants at higher root temperatures, but little reliable evidence appears to be available, especially where plants have been grown in soil.

Riethmann (1933) claimed that stem growth and fruit set largely depend on root temperature and for the variety Schöne von Lothringen the optimal root temperature was approximately 33° C. (91° F.). Went (1944), however, showed that the growth rate of tomatoes was mainly determined by the temperature at which the tops of the plants were grown and that root temperature had little effect upon the growth rate of the plants.

The experiments reported in this paper were undertaken at the suggestion of the British Electricity Authority who provided the electrical soil warming equipment. The aim of the investigation was to determine the effect of maintenance soil warming on the yield of tomatoes grown at normal and sub-normal air temperatures. The term " maintenance warming " refers to the practice of maintaining increased soil temperatures for a period after planting.

MATERIALS AND METHODS.

Glasshouses.

Experiments were carried out in two glasshouses (Nos. 2 and 7), each 102 ft. × 32 ft. and partitioned across the centre into two equal compartments independently heated and ventilated. Glasshouse No. 2 is orientated north-south and the compartments are designated 2A (north) and 2B (south). Similarly, the two compartments in the east-west glasshouse (No. 7) are designated 7A (west) and 7B (east). Both glasshouses are centre ones in blocks having similar orientation and dimensions (John Innes Leaflet No. 10, 1950). These double-compartment houses enabled two different air temperatures to be used in each experiment.

The soil in the two glasshouses is a medium to heavy loam. It had grown tomatoes for five years prior to the experiments but the soil was steam sterilized every year and normal base dressings of fertilizer were applied before planting.

Soil warming equipment.

The soil area of each compartment was divided into four equal sections (quartered). The soil of two of the sections was warmed by means of galvanized iron wires, the other two sections serving as controls. The wires, spaced 6–7 in. apart at a depth of 10 in., carried a low voltage current (15 volts) which was supplied from the

mains (240 volts) through suitable transformers. The warming wires were of such a gauge and length as to give a loading of 5–6 watts per sq. ft. of soil surface.

Soil warming was in operation on certain nights during the period 5 p.m.–9 a.m. The number of nights on which the equipment was used depended on the soil temperature at 6 in. depth as recorded daily at 9 a.m. A target soil temperature was adopted for the warmed sections in each compartment and the warming equipment was in operation each night until the required temperature was recorded at 9 a.m. On the following night the equipment was not used and remained unused until the temperature fell below the required level. The total number of units consumed for each house was recorded on a consumption meter.

Since, under commercial conditions, it is considered unwise to plant tomatoes in soil having a temperature lower than 57° F. (Bewley, 1950), it is normal practice to turn on the heating system some days before planting and to raise the temperature of the soil by conduction from the warmed air. Consequently, this practice was followed for each experiment. Where a low night air temperature of 52° F. was maintained, the control sections tended to fall below the starting temperature of 57° F.; hence the average 9 a.m. temperature in these sections was slightly less than 57° F. Table I summarizes the main details of the experiments in this connection.

TABLE I.

Temperatures maintained, and certain related particulars, in the experiments.

Compartment	Air temperature*		Soil temperature†			Nights in use	Units consumed	Period of warming
	Day	Night	Target	Warmed	Control			
1953								
2A	60° F.	52° F.	62° F.	61·4° F.	56·8° F.	10	397	11 Feb.–2 Mar. (23 days)
2B	65° F.	57° F.	65° F.	64·4° F.	60·9° F.	11	488	
1954								
7A	63° F.	58° F.	70° F.	65·7° F.	58·4° F.	28	1,165	12 Feb.–12 Mar. (28 days)
7B	60° F.	52° F.	70° F.	65·6° F.	56·5° F.	28	1,410	

* Thermostat settings during period of soil warming.
† Recorded at 9 a.m., 6 in. depth.

EXPERIMENTAL.

In conducting experiments of this nature within a limited glasshouse area, it is difficult to arrange an experimental design that will allow complete statistical analysis of the results. In both experiments described in this paper a form of split-plot design was employed but it was impossible to afford sufficient replications to estimate all interactions. Comparisons of the effect of air temperature on cropping were limited to two compartments. Fortunately, the differences in yield between such compartments were generally large for early crops ; they were, indeed, much larger than might be expected to arise from inherent differences between compartments. This is borne out by the similarity of the results obtained when compartments of different orientation and entirely different exposure were used.

Experiment 1.

Seeds of Potentate and Ailsa Craig were sown on 5th January, 1953, and the resultant seedlings pricked out on 16th January into large size (approximately 3 in. diam.) soil blocks made from John Innes Potting Compost No. 1 (JIP1), and grown in a propagating house at a temperature of 60°–63° F. during the day and 55°–58° F. at night. Sturdy plants were thus produced which were planted in permanent positions in compartments 2A and 2B on 9th February. At this stage the first inflorescences were visible in the tips of the plants. Each quarter section of the compartments contained 40 plants (20 Potentate and 20 Ailsa Craig). There were thus 80 plants per soil treatment per compartment. Normal glasshouse methods were used in the cultivation of the plants. Ripe fruit was harvested and recorded at weekly intervals. A summary of the results is given in Table II, where the average weights of ripe fruit per plant are set out for the early crop (first three weeks of picking) and for the total crop.

TABLE II.

Tomato yields (oz. per plant) as affected by soil temperature and air temperature.

Compartment	Early crop*		Total crop	
	Potentate	Ailsa Craig	Potentate	Ailsa Craig
2A (air cool)				
Soil warmed	17·23	1·20	129·93	150·55
Control	20·40	2·35	132·18	152·95
2B (air warm)				
Soil warmed	27·40	7·43	132·75	140·53
Control	31·48	9·48	119·65	141·00

* First 3 weeks' picking.

STANDARD ERRORS.

1. For differences between compartment means :
   Early crop, ±0·50 oz.
   Total crop, ±5·52 oz.
2. For differences between soil temperature means and variety means :
   Early crop, ±1·40 oz.
   Total crop, ±5·13 oz.

For the early crop it will be seen that the differences between warmed and unwarmed plots are small but each difference is in favour of the unwarmed plots. On the other hand, considerably larger differences, all highly significant, are shown in favour of the plants grown at the higher air temperature.

Records for the total crop generally show only small differences between warmed and unwarmed plots. In three comparisons unwarmed plots produced very slightly more fruit, but in the fourth, viz. Potentate in compartment 2B (warm), a difference of 11 per cent. was recorded in favour of soil warming.

Experiment 2.

Seeds of variety Potentate were sown on 28th December, 1953, and the seedlings pricked out on 7th January, 1954, into 3½ in. clay pots filled with JIP1.

In this experiment treatments supplementary to the air and soil temperatures

of the cropping compartments were included. These treatments were confined to the first three-week period after pricking out and were as follows :

(*a*) Artificial illumination *versus* control.
(*b*) Night air temperature of 60° F. *versus* 50° F.

Planting out into the permanent positions in compartments 7A and 7B took place when the plants having the above treatments reached the same stage of development, i.e. when the first inflorescences were just visible in the tips of the plants. The particulars of propagation treatment, in relation to planting in the experimental compartments, were as follows :

Night air temperature 60° F., illuminated ; planted out, 13.2.54.
,, ,, 50° F., ,, ,, 18.2.54.
,, ,, 60° F., not illuminated ; ,, 23.2.54.
,, ,, 50° F., ,, ,, 26.2.54.

Within each soil temperature section there were 24 plants from each of the propagation treatments. These were randomized in six blocks of four plants per plot. Thus there were 96 plants in each soil temperature section and 192 plants per soil temperature treatment. A summary of the results is given in Table III.

TABLE III.

Yields of Potentate tomato (oz. per plant) as affected by propagation treatments and by soil temperature and air temperature in the experimental glasshouse (48 plants per observation).

	Propagation treatments			
	60° F. day, 60° F. night		60° F. day, 50° F. night	
	Illuminated	Not illuminated	Illuminated	Not illuminated
Early crop				
Compartment 7A (air warm)				
Soil warmed	8·21	2·71	7·19	3·54
,, not warmed	12·33	5·56	8·56	4·52
Compartment 7B (air cool)				
Soil warmed	2·63	2·90	2·88	2·08
,, not warmed	3·19	2·63	2·90	2·77
Total crop				
Compartment 7A				
Soil warmed	140·40	128·29	137·50	122·79
,, not warmed	140·83	130·52	140·02	124·79
Compartment 7B				
Soil warmed	126·77	117·25	121·15	113·10
,, not warmed	114·88	116·29	118·06	111·96

STANDARD ERRORS.

1. For differences between compartment means :
   Early crop, ±0·72 oz.
   Total crop, ±2·71 oz.
2. For differences between soil temperature and propagation treatment means :
   Early crop, ±0·43 oz.
   Total crop, ±1·71 oz.

As regards the early crop, Table III shows that, in both compartments, warming the soil failed to increase the crop ; indeed, in every instance except one, plants from the soil-warmed sections cropped later than those not soil-warmed. If all the treatments within these sections are grouped, then the difference is significant ($P<0{\cdot}001$) in compartment 7A but is not significant in compartment 7B.

As in the previous experiment, the different air temperatures operative after planting out produced marked effects, but it is interesting to note that the low temperature in compartment 7B almost completely masked the effects of the treatments given during the propagating stage. Even the large growth differences which were observed at the end of the illumination period were almost entirely lost under conditions of low night temperatures in the plant house. With higher night temperature the various propagation treatments produced the expected responses, except for the unaccountably low average for the plants propagated at 60° F. day, 60° F. night and not illuminated.

The figures for the total crop of ripe fruit showed that soil warming did not induce a larger crop for any propagation treatment followed by growth in compartment 7A (63° F. day, 58° F. night), all differences being in favour of the unwarmed sections, though they are not significant. Of the plants grown in compartment 7B (60° F. day, 52° F. night), those in the soil-warmed sections always outyielded the controls. The largest difference (10%) occurred when the plants were propagated at 60° F. day and night and illuminated. However, if all the propagation treatments within these sections are grouped, then the difference is not significant.

DISCUSSION.

Under the conditions of these experiments, maintaining the soil temperature 5–9° F. above that of the control, for a period of up to one month after planting, did not result in significant increases in yields of tomatoes. This was true irrespective of the air temperature at which the plants were grown. In contrast, considerable differences in the early yield resulted from differences in the air temperature during the first month after planting, a low air temperature causing a reduction in the crop.

These results agree with those of Went (1944) who concluded that growth rate and fruiting are governed primarily by the temperature at which the tops of the plants are growing. Only when plants are growing under sub-optimal conditions does the root temperature materially affect the growth rate and the magnitude of the crop. This is the basis of Went's explanation of Riethmann's (1933) findings. Went, in his experiments, records growth rates up to 27·5 mm./day and estimates that the growth of Riethmann's plants was only 14 mm./day.

If an attempt is made to apply this principle to the results of thes eexperiments, it will be seen that, for the total crop in Experiment 2 (Table III), soil warming was most effective when applied under conditions of low air temperature (compartment 7B). This result could be attributed to an increase in root temperature under sub-optimal growth conditions, i.e. low air temperature.

In this connection it is interesting to note a similar instance (Lawrence and Calvert, 1954) in which artificial illumination had its greatest relative effect when the method of propagation was poorest, each successive improvement in method resulting in a smaller relative increase in yield from illumination.

It should not be assumed that, because maintenance warming might prove most

ESTABLISHMENT OF SEEDLINGS IN THE FIELD

I. INFLUENCE OF SOWING DEPTH ON SEEDLING EMERGENCE

By WALTER HEYDECKER

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In order to produce a stand of plants, sowings in the field have to pass through the following stages : (1) germination, i.e. the inception of rapid metabolic activity within the seed, resulting in perceptible growth of the embryo, first the radicle and then the aerial parts appearing from inside the testa ; (2) underground elongation of the seedling, relying on its food reserves ; (3) emergence* of the aerial parts from the soil ; and (4) the beginning of photosynthesis, resulting in dry weight increase of the plant. From this stage onward, normally functioning seedlings may be considered established, although plants become progressively more securely established with increasing age.

Research results on the subject of seed germination, showing the complex relationship between seed and environment during this process, have been summarized by Crocker and Barton (1953). Laboratory experiments in the course of the present work have confirmed that changes in any one of the following environmental factors may considerably modify the percentage and/or speed of emergence from the soil : temperature, soil moisture, soil compression, soil particle size, composition of the atmosphere surrounding the seed and protection of the seed against pathogens by soil sterilization and/or chemical seed dressing. The interaction of these factors, and the profound influence exerted on them by the soil type, were also indicated. Seeds of different species often behaved very differently under identical environmental conditions.

Arising from the laboratory work, a field experiment was designed to ascertain the effects on emergence, either independently or in interaction, of sowing on different dates and at different depths. Since changes of temperature and moisture in the seed bed between sowing and establishment are usually unpredictable at the time of sowing, it appeared desirable to show whether choice of sowing depth could minimize the effects of adverse conditions that might develop in the seed bed after sowing.

Review of the literature.

Moore (1943) showed that certain small-seeded agricultural crops emerged best under a variety of conditions when sown at 10–20 mm. depth. Decker (1946) reported poor survival of lupin sown at a depth of 50 mm., due to attack of pathogenic fungi. Andrew (1953) found that, when the initiation of germination was followed by a 10-day period at 10° C., sweet corn seed sown 100 mm. deep gave only 49 per cent. emergence as compared with 83 per cent. for seed sown 25 mm. deep, and Warren (1950) showed that the stand and subsequent yield from bush lima beans

* The term " emergence " is occasionally used in botanical terminology to describe the final stages of (1). Agronomically it usually signifies (3), and this meaning will be adhered to throughout this work.

suffered when the seeds were sown 100 mm. deep as compared with sowing depths of 25 mm. or 50 mm., these latter not differing in their effects. Elze (1949), working with citrus seed, found that the optimal depth was 10 mm. but that for practical purposes sowing at 20–30 mm. deep was advisable, presumably in the main because of risk from drought. Reynolds (1950) enumerated the factors that might influence optimal sowing depth for peas ; though differences in yields from 38 mm. and 75 mm. sowing depths were not significant, yields from 38 mm. were generally no worse and sometimes better than those from 75 mm. deep sowings. Holmberg and Minges (1952) found little difference in emergence time of tomato seed sown in the open ground at depths between 13 mm. and 88 mm. but did not state whether the emergence percentage was affected. On the same subject, Parfenov (1954) stated that tomatoes of the determinate type are best sown at 30 mm., and those of the taller indeterminate type at 50 mm. depth. Torres *et al.* (1952) recommended that for good germination manila hemp (*Musa textilis*) should be sown at 20 mm. or less, and Jorritsma (1953) found that sugar beet gave highest percentage emergence at 20–30 mm. depth but that, up to a point, root weight increased with greater sowing depth. Wofford and Dexter (1955) also reported on work showing the importance of sowing depth in obtaining a good stand of sugar beet. Black (1955), working with subterranean clover, showed that the reduction of food reserves in the cotyledons between incipient germination and emergence from the soil is proportional to the depth of sowing.

MATERIALS AND METHODS.

The soil chosen was a well-drained sandy loam composed of somewhat stony glacial drift overlying Keuper marl. It was in good heart and no fertilizers or manures were applied during the experimental period. After initial ploughing during the previous winter, no deep cultivation was carried out.

In view of the possible advantages arising from the use of seed units of standard size and shape in conjunction with precision drills, lettuce and carrot seed coated* with montmorillonite were included in the experiments in addition to plain seed (Table I). Unfortunately, coated and uncoated seed of identical variety and history

TABLE I.

Seeds used in the experiments.

Species	Year of harvest	Plain or coated	Variety	Germination percentage (laboratory tests)
Cabbage	1952	Plain	Tozer's June Giant	96
Lettuce	1951	Plain	Tozer's Cobham Green	97
Lettuce	?	Coated	Filcoat Imperial No. 615 Lot C No. 899	95
Carrot	1952	Plain	Tozer's Ideal	75
Carrot	?	Coated	Filcoat Long Imperator Lot C No. 314	85
Onion	1952	Plain	Harrison's Giant Zittau	65

* The definition accepted officially in the U.S.A. (Hay, 1949) is adopted here : coating refers to the covering of individual seeds ; pelleting to the inclusion of a number of seeds within each " parcel ".

was not obtainable, and no comparison can therefore be made between results from coated and uncoated seed.

All the uncoated seeds were treated with thiram dust (0·1 gm. Fernasan A per 85 gm. seed) on 20th March, 1953. Various active soil-borne pests, notably surface caterpillars (*Agrotis* sp.), were found at the beginning of July 1953 and all plots were treated with benzene hexachloride (50 per cent. dispersible crude BHC powder, one ounce per three gallons of water and approximately one fluid ounce per foot run of row).

In laboratory experiments cabbage seed proved the most, and lettuce seed the least, capable of germinating in soil of low moisture content. Hence, for uncoated lettuce seed, two series were run, in one of which the top half-inch of soil was removed before the drill was drawn whilst in the other no surface soil was removed. In the latter, when the surface soil was dry at sowing time, some dry soil particles unavoidably ran into the bottom of the drill as this was opened.

Sowing technique.

To determine and replicate accurately the depth of sowing, a simple tool—hereafter called the "sowing board"—was constructed (Fig. 1). This consisted essentially of two planks, approximately six feet long, laid flat and held together by a cross piece at either end in such a way that the gap between them was wider at one end than at the other. Using a triangular piece of wood, held at right angles to the two planks, with its point projecting into this widening slot and with its sides always in contact with their inside edges, the operator was able to draw a drill which was shallow at the narrow end and deepened evenly towards the wide end of the slot. To prevent wear, the sides of the "triangle" and the inside edges of the planks were clad with steel strips.

For each sowing operation the soil was levelled with a rake, the sowing board placed in position, and some of the soil at the wide end of the slot removed with a trowel. Standing on the two planks, the operator then drew the triangle from the narrow towards the wide end, with short overlapping strokes, so that a smooth, evenly graded drill was produced. Any stones encountered by the point of the triangle were removed. Intervals of 10 cm. were marked off along the board to indicate sowing stations in the drill. Eighteen sowing depths, ranging from 0 to 95 mm. and progressing in steps of 5–7 mm., were thus obtained. Five seeds were sown in the bottom of the drill at each of the stations marked on the sowing board. The drill was then refilled with soil which was replaced in its original position as far as possible. At the end of this operation a slight ridge of soil remained between the two planks. By moving the sowing board several times to and fro at right angles to the direction of the drill, the operator was able to settle the soil in the drill without consolidation, causing the soil ridge along the drill to disappear and producing a smooth and level surface finish.

A randomized block layout with four replications was used. Each of the four major blocks contained seven secondary blocks, sown on different dates during spring and early summer of 1953. Each secondary block contained 18 plots, i.e. stations as marked on the planting board, representing the 18 sowing depths.

Emergence of aerial parts from the soil was taken as the criterion of success. Counts were made at intervals in order to obtain a measure of the speed of emergence as well as the final score. Most of the earlier sowings were allowed to grow until the plants matured, when sizes and weights were recorded.

RESULTS.

Effects of sowing date.

Brief notes on meteorological and soil conditions existing on the seven sowing dates are presented in Table II.

TABLE II.

Sowing dates and meteorological conditions.

Number of sowing	Sowing date 1953	Temperature extremes for 14 days after sowing, 1 cm. below bare ground		Rainfall (in.)		Moisture condition of soil at time of sowing
		Min. °C.	Max. °C.	1 week before sowing	2 weeks after sowing	
I	20–21 Mar.	1	20	0·0	1·72	Moderately dry.
II	15–17 Apr.	1	29	0·21	0·82	Moderately moist.
III	1–2 May	2	27	0·60	0·42	Moist, gradually drying.
IV	19 May	6	33	1·37	0·83	Rather wet, preceded and followed by heavy rain.
V	18–20 June	10	32	1·51	1·05	Rather wet, preceded and followed by heavy rain.
VI	8–10 July	12	30	0·04	1·11	Dry, then heavy rain.
VII	24–25 July	10	36	0·28	0·16	Rather dry, followed by drought.

Speed of emergence. This increased in all species as the season advanced from March to June.

Total emergence. The results are summarized in Table III. Emergence of all species was uniformly good on date III. Apart from this, significant differences occurred between different species sown on the same date, and within species for different sowing dates. It was not possible to establish correlations between the meteorological data and the emergence of the various species. Generally, spring conditions, though less conducive to rapid emergence, produced a higher percentage of successes than summer conditions, but this difference was not statistically significant, and one sowing in summer (No. VII), followed by severe drought, ultimately resulted in a satisfactory stand of carrot, in contrast to that of all other species.

Effects of sowing depth.

Independence of effects of date and depth. Figs. 2A and 2B show the effects of sowing depth on the emergence of carrot and onion. In these composite histograms, differences in actual numbers emerging from successive sowings were eliminated by expressing, for each date, the highest number emerging as 100 and the numbers emerging at the other sowing depths as percentages of the highest number.

The possibility of interaction between sowing date and optimal sowing depth was tested, but no relationship was found to exist which could be predicted either by the calendar date or by the conditions of soil and climate prevailing on the day of sowing. (See statistical report.)

Thus it became possible to determine the optimal sowing depth for the crops

TABLE III.

Variation of emergence (depths 5–56 mm.) with sowing date, shown as a percentage of the laboratory germination test values. (Data are based on 4 blocks × 10 depths × 5 seeds per date.)*

Seed	Possible germination (expected under ideal conditions) %	Sowing date 1953							Standard error (a)†	Grand mean	Standard error (b)†
		I March 20–21	II April 15–17	III May 1–2	IV May 19	V June 18–22	VI July 8–10	VII July 24–25			
Cabbage	96	75·0	81·0	76·5	78·5	69·5	56·5	54·5	4·3	70·2	1·6
Lettuce (plain)	97	62·5	66·5	64·5	63·5	56·0	41·0	47·0	4·1	57·3	1·6
Lettuce (plain, dust tilth removed) ..	97	73·0	73·0	71·0	64·0	67·5	43·5	48·0	3·9	62·9	1·5
Lettuce (coated)	95	64·5	69·0	67·5	57·0	60·0	32·0	52·0	4·5	57·4	1·7
Carrot (plain) ..	75	52·5	55·5	73·5	41·5	35·5	50·5	62·0	4·9	53·0	1·9
Carrot (coated)	85	54·5	56·0	70·5	49·5	52·5	46·0	78·0	5·2	58·1	1·9
Onion	65	63·0	72·5	67·5	50·0	64·5	51·0	57·0	5·1	60·3	1·9

* This range of depth was chosen as representative of depths likely to be encountered under conditions of field cultivation.

† For each kind of seed, standard error (a) refers to the emergence percentages for separate sowing dates; standard error (b) to the grand mean for all sowing dates.

PLATE I

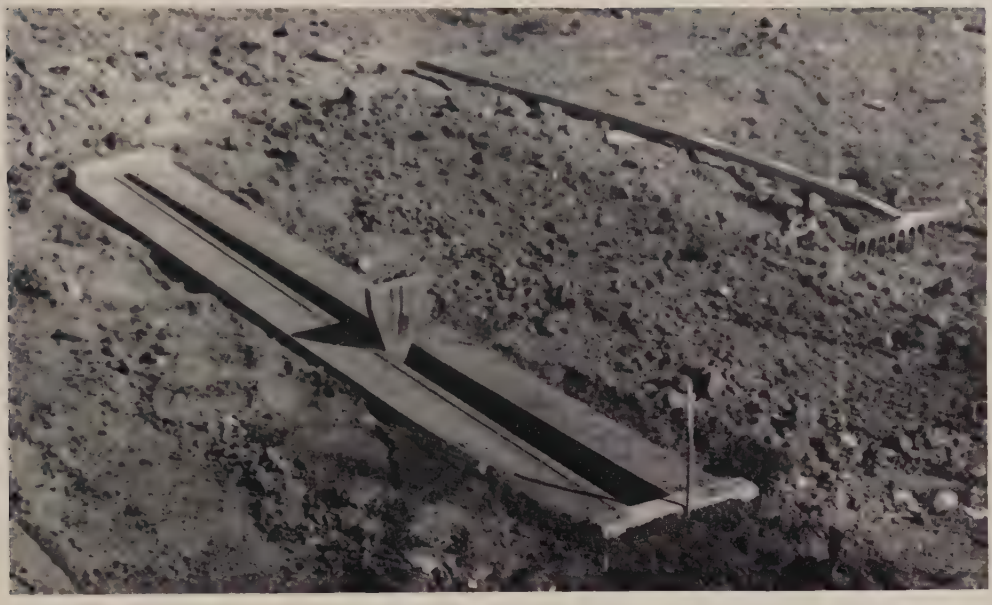


Fig. 1.
Sowing board : simple device to produce a gradually sloping drill.

Fig. 4.
Stand of cabbage var. June Giant six weeks after sowing (date III).
Sowing depths: label in front=0, label at back=95 mm.

[face p. 80

TABLE III.

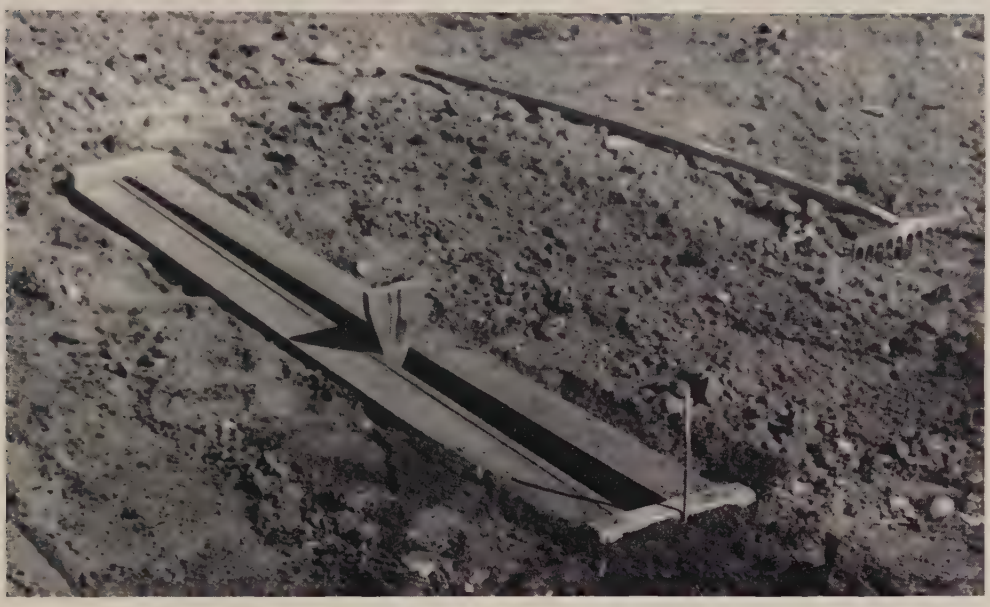
Variation of emergence (depths 5–56 mm.) with sowing date, shown as a percentage of the laboratory germination test values. (Data are based on 4 blocks × 10 depths × 5 seeds per date.)*

Seed	Possible germination (expected under ideal conditions) %	Sowing date 1953							Standard error (a)†	Grand mean	Standard error (b)†
		I March 20–21	II April 15–17	III May 1–2	IV May 19	V June 18–22	VI July 8–10	VII July 24–25			
Cabbage	96	75·0	81·0	76·5	78·5	69·5	56·5	54·5	4·3	70·2	1·6
Lettuce (plain)	97	62·5	66·5	64·5	63·5	56·0	41·0	47·0	4·1	57·3	1·6
Lettuce (plain, dust tilth removed) ..	97	73·0	73·0	71·0	64·0	67·5	43·5	48·0	3·9	62·9	1·5
Lettuce (coated)	95	64·5	69·0	67·5	57·0	60·0	32·0	52·0	4·5	57·4	1·7
Carrot (plain) ..	75	52·5	55·5	73·5	41·5	35·5	50·5	62·0	4·9	53·0	1·9
Carrot (coated)	85	54·5	56·0	70·5	49·5	52·5	46·0	78·0	5·2	58·1	1·9
Onion	65	63·0	72·5	67·5	50·0	64·5	51·0	57·0	5·1	60·3	1·9

* This range of depth was chosen as representative of depths likely to be encountered under conditions of field cultivation.

† For each kind of seed, standard error (a) refers to the emergence percentages for separate sowing dates ; standard error (b) to the grand mean for all sowing dates.

PLATE I



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[face p. 80

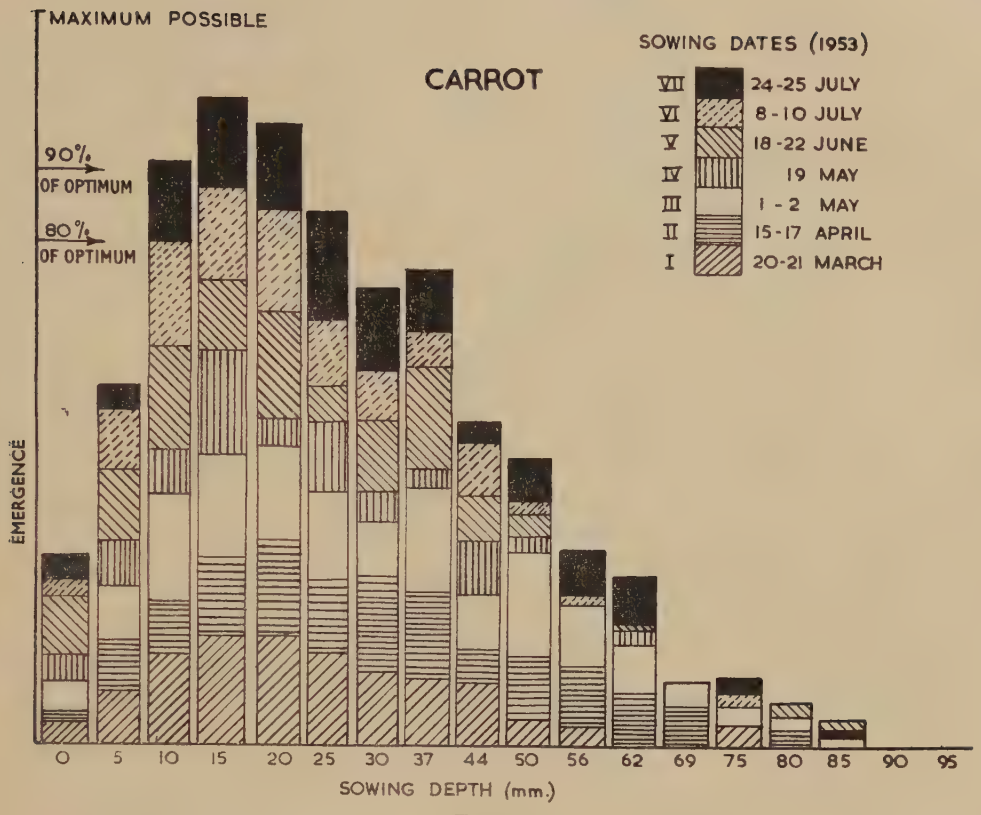

FIG. 2A.

Emergence of carrot expressed as percentage of optimum number of seedlings emerging on individual sowing dates.

tested, independently of sowing date. It should be stressed that the conclusions reached are based on data obtained on a single soil type during a single season which included, however, a wide variety of weather conditions.

Determination of optimal sowing depth. For each kind of seed the ranges of optimal depths which were established for individual dates overlapped, and the overlapping part of the optimal depth range may be called the " safe " sowing depth for this kind of seed under the varied and unpredictable environmental conditions of the experiment. To establish these safe sowing depths, curves were fitted to the recorded data, sowing dates being regarded as replicates in time. These results are presented in Fig. 3. The safe range was taken to include those depths at which emergence amounted to at least 90 per cent. of the largest number on the curve. For the " probably safe " range, limits were widened to include depths where at least 80 per cent. of the largest number could be expected (see Table IV).

In the following report on individual species, the same convention is employed.

Optimal sowing depths for individual species. Cabbage seed was less susceptible to the detrimental effects of deep sowing than any other kind of seed, since the strong

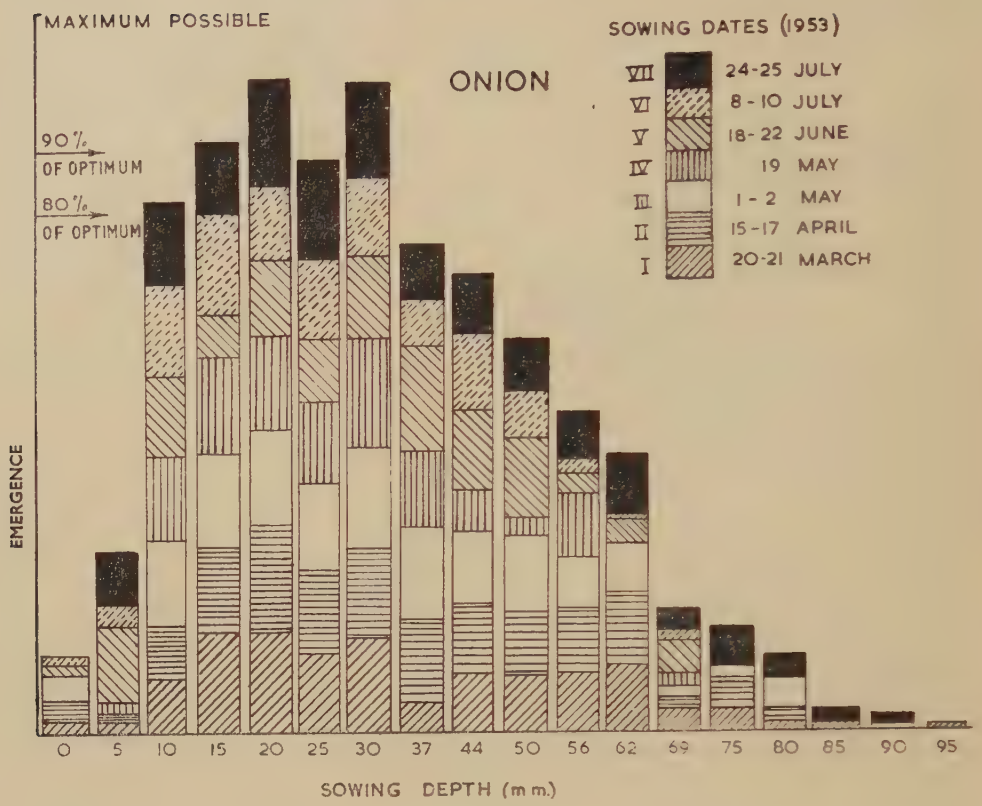

FIG. 2B.

Emergence of onion expressed as percentage of optimum number of seedlings emerging on individual sowing dates.

TABLE IV.

Optimal sowing depths.

	"Safe" (90% of maximum) emergence	"Probably safe" (80% of maximum) emergence	Statistical optimum $P<0.05$
	mm.	mm.	mm.
Cabbage	15–39	11–47	15–50
Lettuce (plain)	14–30	10–35	10–30
Lettuce (plain, dust tilth removed)	10–30	6–35	10–30
Lettuce (coated)	9–25	5–31	10–25
Carrot (plain)	13–26	10–30	10–30
Carrot (coated)	12–28	8–33	10–37
Onion	15–33	11–41	10–37

"Safe" limits are derived from the curves defined in Table V and shown in Fig. 3.
The statistical optimum is based on the experimental data, irrespective of curves fitted.

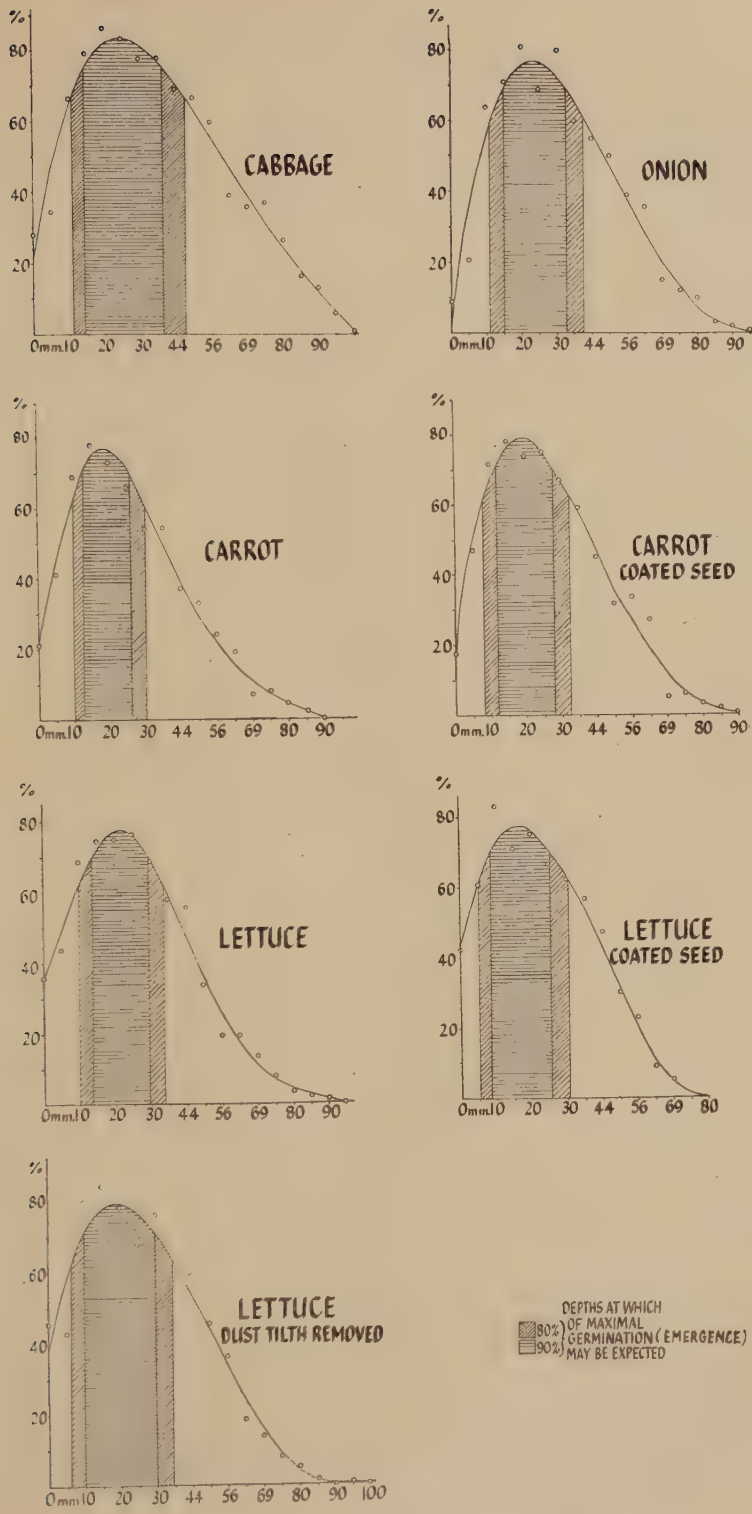

FIG. 3.
Effect of sowing depth on emergence of vegetable seedlings.
Ordinates: total percentage emergence of viable seeds.
Abscissae: sowing depths (mm.).

hypocotyls of cabbage seedlings were able to penetrate the mechanical obstruction offered by even thick layers of soil. On the other hand, sowing at less than 15 mm. depended for success on rainfall following fairly closely upon sowing. Good results were obtained from any depth in the range 11 to 47 mm. (probably safe) but the safe range was 15–39 mm. (0·6–1·6 in.). Emergence of cabbage seedlings from sowings made at different depths is shown in Fig. 4.

Lettuce hypocotyls from deeply-sown seeds were less successful in carrying the cotyledons to the surface and were often found to be laterally compressed and distorted, the structure of the hypocotyl being much more fragile than in cabbage. Emergence successes diminished steeply as sowing depth increased beyond 30 mm., but emergence from a depth of 10 mm. was relatively more likely than with cabbage ; the safe depths were 14–30 mm. (0·5–1·2 in.). Removal of the dust tilth neither significantly increased total emergence nor modified the optimal sowing depth ; according to the curve fitted, shallow sowing may be safer than when the dust tilth is retained.

For coated lettuce seed the safe depth was 9–25 mm. Few lettuce seedlings were able to emerge from depths of 50 mm. or more, yet some of the heaviest heads were ultimately produced by such plants. This was presumably due to reduced competition but it tended to show that deep sowing does not necessarily have an adverse effect on the yield ultimately produced per plant.

Carrot emergence dropped off steeply at depths greater than 30 mm. and the safe sowing depth was 13–26 mm. (0·5–1·0 in.).

For coated carrot seed the safe depth was 12–28 mm.

It is interesting to note that carrots harvested from deeply-sown seeds were consistently longer than those from shallower sowings ; all the hypocotyl became part of the edible root ; individual root weight was thus greater but this was far from compensating for the low emergence percentage.

Onion, though generally supposed to emerge better when sown very shallow, emerged even less well from 5 mm. depth than any of the other species. A depth of 10 mm. was much more favourable. Emergence of onion seedlings from depths of 50 mm. or more was superior to that of lettuce and carrot. The safe sowing depth was 15–33 mm. (0·6–1·3 in.).

At greater sowing depths, bulbs formed with some delay and below ground ; although they attained individual weights as good as those from shallow sown seeds, there were increased difficulties in drying-off prior to harvesting and in harvesting.

Effect of sowing depth on crop performance. Sowing shallower than at the optimal depth not only retarded and reduced emergence in dry soil but occasionally also delayed the growth of seedlings before they could achieve a firm root hold. Deep sowing, apart from reducing the number of seedlings successful in establishing themselves, always delayed their emergence. At optimal depths emergence was both consistently high and consistently rapid.

All the types of crop tested were quite capable of ultimately producing satisfactory yields from plants which emerged from great depths. Nevertheless, competition with weeds and with neighbouring plants which have originated from shallower sown seeds is generally likely to put a plant from a deeply-sown seed at a serious disadvantage. In these experiments, seedlings emerging a few days later than their immediate neighbours could almost invariably be recognized throughout their life by their dwarfed growth.

DISCUSSION.

Sowing at the optimal depth in the field did not necessarily ensure the best stand of which seed was capable, as shown by a comparison of total numbers which emerged from any particular depth on different sowing dates. Conversely, there was some latitude in the range of sowing depths from which optimal seedling emergence occurred under any one set of conditions ; however, environmental changes subsequent to sowing not being predictable, the depth from which optimal emergence was likely to occur under a large variety of conditions was much more limited.

Results obtained from sowing on seven dates during the season of 1953 show that a striking similarity in pattern exists when emergence percentages of the different crops at various depths are compared. For all the kinds of seed and on all the sowing dates, optimal emergence occurred from fairly shallow sowing depths. Emergence rates fell off only gradually as the depth was increased from the optimal, but steeply when it was reduced. With all the species tested—cabbage, lettuce, carrot and onion—under all conditions encountered in the experiment, the curves fitted showed that depths of 11–30 mm. were " probably safe " and depths of 15–25 mm. (0·6–1·0 in.) comprised a " safe " depth range from which seedlings emerged as well as, or better than, from any other depths. The latter range might thus be regarded as a safe standard depth at which to aim in sowing these kinds of vegetables under English summer conditions in soils of the type used in this experiment.

It might be expected that the optimal sowing depth would differ on other soil types but, in a series of laboratory experiments with a well-structured clay loam, a depth of 17 mm. was found for all these species to be at least as favourable as, and sometimes better than, shallower (12 mm.) or greater (25 mm.) depths. The loam was used at high and low moisture tensions, at a constant temperature of 60° F. and in a still atmosphere. These results suggest that a sowing depth of 17 mm. may prove to be suitable for a considerable range of environmental conditions, largely irrespective of soil and climate.

In the field, deviation from the safe depth range may result in improvement in some factors being largely cancelled out by deterioration in others. For instance, deeper sowing may improve seedling anchorage and increase the probability of adequate moisture supply but it would also increase mechanical obstruction to the emerging seedling and the possibility of inferior aeration, which in turn might delay emergence and increase the danger from microbial attack to which long hypocotyls appear to be more susceptible than shorter ones (Hanf, 1943). On the other hand, sowing too shallow may prevent successful germination, despite excellent aeration of the soil, by subjecting the seed to drought conditions. For instance, an optimal depth of 3–6 mm. has been found for sowings in seed boxes in moistened compost covered with glass ; under such uniformly moist conditions seeds require a layer of covering material only deep enough for anchorage (Majmudar, 1955). In the field, however, the top 10 mm. of soil is likely to dry out during rainless periods and thus to provide insufficient moisture for seed germination.

Under the conditions of this experiment, two-thirds to three-quarters of the viable seeds sown at a safe depth could be relied upon to produce seedlings under a wide range of conditions. Although this figure may appear low it compares favourably with stands often obtained in the field. These results suggest that the depth of sowing obtained from mechanical seed drills should be accurate to within

± 7 mm., this tolerance representing the narrowest safe depth range (that of carrot) established so far.

SUMMARY.

1. A technique was developed for sowing seeds accurately at a range of predetermined depths.

2. Cabbage, lettuce, carrot and onion seeds were sown in a sandy loam at 18 depths, ranging from 0 to 95 mm., on seven dates in spring and summer, 1953.

3. Sowing date and sowing depth influenced emergence percentage significantly and independently.

4. The effect of sowing on any particular date proved unpredictable, although a seasonal trend was apparent ; more seedlings generally emerged from spring than from summer sowings.

5. A range of optimal sowing depths was found for each kind of seed.

6. Despite the heterogeneous nature of the types of seed used (i.e. different sizes, presence or absence of endosperm, di- or mono-cotyledons and coated (" pelleted ") seeds), all the optimal depth ranges overlapped over the range of 15–25 mm. (0·6–1·0 in.) for the seeds tested under the conditions of this work. The need for the provision of accurate depth adjustment in mechanical seed drills is stressed.

ACKNOWLEDGMENTS.

This work was suggested by Dr. J. P. Hudson, Head of the Department of Horticulture, University of Nottingham, to whom I wish to express my gratitude for his constant interest and helpful criticism.

I also wish to thank Dr. G. H. Jowett and Miss Hilda M. Davies, Department of Mathematics, University of Sheffield, for carrying out the statistical analysis of the data.

The technical assistance received from Miss D. J. M. James throughout this work is gratefully acknowledged.

I am indebted to the Filtrol Corporation, 3250 E, Washington Boulevard, Vernon 23, California, U.S.A., for the gift of the " Filcoat " brand coated seeds used in these experiments.

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APPENDIX

STATISTICAL ANALYSIS OF DATA

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Department of Mathematics, University of Sheffield

Analysis of variance, carried out for depths from 5 to 56 mm. inclusive, showed that the proportion of seeds emerging depended both on depth and date of sowing and that these effects were independent of one another. Only in the case of lettuce (plain seed) was there a (highly significant) difference between replications in space. Variation of emergence with sowing date is presented in Table III. Curves were fitted to variations of mean proportions emerging from increasing sowing depths, utilizing all points up to (but for logarithmic values not including) the first value of zero emergence. It was not found possible to fit all cases by curves of the same mathematical family, though their shapes were all fairly similar. The curves were polynomials fitted to the variation of either mean proportion of emergence or the logarithm of these values with the depth (Fig. 3 ; Table V).

No more satisfactory single curves than those fitted here have been found (including " lettuce, dust tilth removed " where the fit is not really satisfactory) ; however, other types of curves may give an equally good fit, yet may have their maximum values corresponding to different depths.

An analysis of the data was therefore performed to establish ranges of optimal sowing depths ($P<0{\cdot}05$) for the individual kinds of seed under the conditions of this experiment. This was done as follows :

On each graph, the highest observed value and the second highest observed value on the right were tested by χ^2 for significant difference of level. Then the

TABLE V.

Variation of emergence with depth.

	Curve	Maximum value of curve	Depth which gave actual maximum number of emerging seedlings
		mm.	mm.
Cabbage	Quartic	24·5	20
Lettuce (plain)	Antilog. of cubic fitted to the logarithms	22·0	25
Lettuce (plain, dust tilth removed)	Quartic	19·5	15
Lettuce (coated)	Antilog. of cubic fitted to the logarithms	17·0	10
Carrot (plain)	Antilog. of quartic fitted to the logarithms	18·5	15
Carrot (coated)	Quartic	19·0	15
Onion	Quartic	22·5	20

second highest observed value on the left was included and the three values were tested for difference of level. This procedure continued, taking points from alternate sides until a significant difference was obtained on one side ; after which, points on the other side only were taken until a significant difference was obtained on that side. The results are included in Table IV.

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TWO METHODS FOR THE BIOASSAY OF AUXINS IN THE PRESENCE OF GROWTH INHIBITORS

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INTRODUCTION.

Auxins are defined as substances which stimulate cell extension in plants and the large majority of techniques for their detection and measurement are based on this characteristic property. Tests of this type include the coleoptile cylinder test devised by Bonner (3) and subsequently modified by other workers (15, 2, 5), the split pea test (18), and the pea root test recently described by Audus and Thresh (1). While such methods are capable of giving excellent results in experiments employing solutions of pure synthetic growth substances, difficulties are often encountered when they are applied to the study of naturally occurring auxins in plant extracts. These difficulties arise from the fact that such extracts invariably contain, in addition to auxins, substances inhibitory to cell extension growth which partially or completely mask the activity of auxins in biological assays. Although little is known at present regarding the chemical nature of these naturally occurring inhibitors, they would appear to fall into two general groups : (1) substances, probably of a phenolic nature, which when concentrated in the extract exert a toxic action on the test object, damaging the tissue and thereby preventing it from responding to the auxin, and (2) true anti-auxins which, by blocking some essential reaction in the chain of processes involved in growth, reduce the activity of auxin in the test. In partially purified ether or water extracts of plant tissues, the combined effect of the various inhibitors present usually outweighs that of the auxins, so that the net effect of such extracts on the growth of coleoptile sections or similar test objects is one of inhibition. Exceptions are sometimes found with seeds or other tissues particularly rich in auxin, extracts of which may give a positive response in cell elongation tests (9). Even here, however, it is not always clearly recognized that what is measured by the test is not the auxin content of the tissue, but the net effect of auxins less that of inhibitors.

By the technique of paper chromatography, now widely used in auxin studies, it is often possible to effect a separation of the auxins and inhibitors in plant extracts, so that each may be assayed individually in the biological test. Where, however, as often occurs, an extract contains several different auxins and inhibitors, complete separation on paper is often difficult to achieve. Auxins and inhibitors whose R_F values differ by less than 15 per cent. usually give overlapping spots on one-directional chromatograms and consequently show mutual interference in biological assays based on cell elongation response. Occasionally the presence of an auxin on the chromatogram may be completely masked by an inhibitor having the same R_F value.

It has been observed that the inhibitors which commonly occur in plant extracts are primarily inhibitors of cell extension, and that they seem to have little or no effect

on other types of auxin response, such as induction of parthenocarpy, prevention of abscission and stimulation of rooting. Hence, by using tests based on such responses, rather than on extension growth, it is possible to assay auxins in plant extracts, even in the presence of inhibitors, and often to obtain a much clearer picture of the distribution of auxins on paper chromatograms.

Relatively few methods of auxin assay which depend on responses other than cell extension have been described in the literature. Amongst these may be mentioned Went's " rhizocaline " test (17), based on the ability of the substance to induce root formation on etiolated pea epicotyls fed with sucrose, the *Ageratum* rooting test developed by van Raalte for use in tropical countries (12), and the tomato ovary test (8). In this paper two further methods for the bioassay of auxins are described : one of these is based on the ability of auxins to delay the abscission of de-laminated petioles of *Coleus* ; the other on their activity in stimulating the formation of root primordia on the hypocotyls of bean seedlings.

THE COLEUS ABSCISSION TEST.

In many plants removal of the leaf blade induces the formation of an abscission layer at the base of the petiole. In *Coleus blumei* this layer develops very rapidly, and for this reason *Coleus* has been widely used as an experimental plant in abscission studies. In 1918 Sampson (14) used this plant for following the chemical changes accompanying abscission, and later Myers (10) studied the development of the absciss layer and was able to establish a correlation between natural leaf auxin and petiole abscission. Myers also showed that the application of growth substances to the de-bladed petioles would delay their abscission. More recently Wetmore and Jacobs (19) have confirmed Myers' observations on the development of the abscission layer in *Coleus* and on the relation between leaf auxin and abscission. Rossetter and Jacobs (13) have further observed that the presence of intact leaves on a *Coleus* shoot will promote the abscission of nearby de-bladed petioles. In the present work, interference from neighbouring leaves has been overcome by using excised nodes of *Coleus* maintained in a moist chamber at constant temperature. Under these conditions it has been shown that a quantitative relationship exists between the amount of auxin supplied to the petiole and the delay in abscission which follows, and that this reaction can be used as the basis of an auxin assay method.

Method.

As in all biological assay methods, uniformity of the test material is of prime importance. The *Coleus* plants used in these experiments were grown from cuttings under ordinary glasshouse conditions. In such material there is a seasonal variation in sensitivity, which could probably be eliminated by growing the plants under controlled conditions of light, temperature and day-length. The red-leaved variety Corunna has been most generally used, but several other ornamental forms are equally suitable. A few varieties have proved to be unsatisfactory owing to the difficulty of keeping them vegetative, or because of the unusually long time required for the development of the abscission layer. Growing conditions should be such as to maintain moderately vigorous vegetative growth and long internodes, but excessive nitrogenous fertilization should be avoided as this tends to delay petiole abscission.

For the test the required number of vegetative shoots, each about 12 inches long,

are cut from the plant. Such shoots typically carry about 8 pairs of expanded leaves, of which numbers 4 to 7 (numbering from the apex) are suitable for use, in that they respond in a uniform manner to applications of growth substances. These four nodes are isolated by cuts made 1 cm. above and below the node, the laminae and axillary buds removed, and the petioles cut with a razor blade parallel to, and at a point 4 mm. from, the stem. Finally the stem piece is split vertically, as shown in Fig. 1,

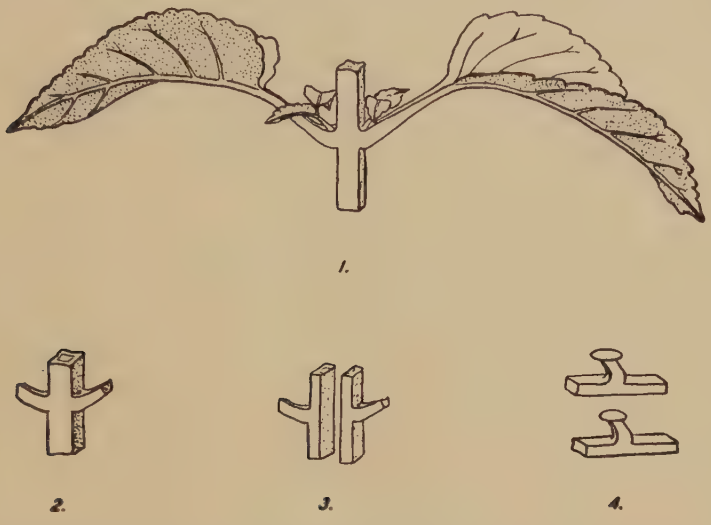

FIG. 1.

Method of preparing *Coleus* nodes for abscission test. (1) Node with attached leaves ; (2) axillary shoots removed, petioles cut to 4 mm. ; (3) stem piece split vertically ; (4) filter paper discs containing auxin or plant extract applied to cut surface of petiole.

and the two portions of half-stem, each with an attached petiolar stub, are placed on moist filter paper supported on a glass sheet in a large dish (Fig. 4). After application of the treatments, the dish is covered with another sheet of glass lined with wet filter paper and placed in an incubator at 25° C. Records are taken at daily intervals after pressing each petiole lightly with a needle to determine whether abscission has occurred.

In the earlier experiments application of auxin to the cut end of the petiole was made in lanolin. This was found unsatisfactory ; not only was quantitative application difficult, but treating the cut end with lanolin frequently induced rotting of the petioles. A more satisfactory method was found to be the use of small discs of filter paper impregnated with known amounts of auxin. The discs, 0·5 cm. in diameter, are punched from a sheet of Whatman No. 1 paper with an ordinary paper punch. Each disc is impaled on the point of a pin, which is then supported in an inverted position by pressing the head into a block of modelling clay. Thus supported, the discs are impregnated with auxin by placing on each a single drop of alcoholic solution delivered from a micro-syringe, the strength of solution being adjusted so that each drop contains the required amount of auxin. After drying, the impregnated discs are stored in a cool dark place in corked tubes until required. Immediately

before application to the petioles they are dipped momentarily in distilled water. At 25° C. absorption of the auxin from the moist filter paper is rapid. Experiments in which the discs were removed after various periods of time showed that there was little further absorption after six hours.

Dosage/response relationships.

Typical dosage/response curves for three synthetic growth substances are shown in Fig. 2, from which it will be seen that, within limits, the number of days' delay in

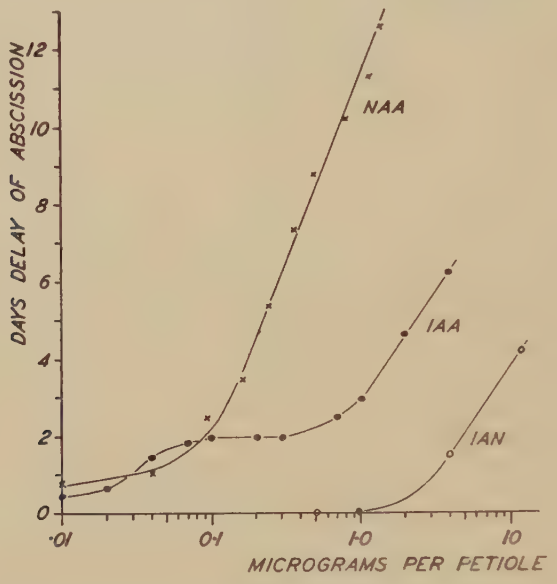

Fig. 2.

Relationship between amount of growth substance applied per petiole and delay of abscission for α-naphthaleneacetic acid (NAA), indoleacetic acid (IAA), and indoleacetonitrile (IAN).

abscission is proportional to the logarithm of the amount of growth substance applied. For α-naphthaleneacetic acid (NAA) the smallest amount which can be detected is of the order of 0·01 μg., but between 0·01 and 0·1 μg. per petiole the test is relatively insensitive. The useful range for bioassay lies between 0·1 and 1·0 μg. per petiole. Within this range the sensitivity of the test is high and the log. dose/response relationship strictly linear. Phenoxy- and naphthyloxy- growth substances also show high activity in this test, whereas indoleacetic acid (IAA) is considerably less active. Although amounts of IAA as low as 0·05 μg. can be detected, the sensitive range over which the test is useful for bioassay purposes lies between 1·0 and 10·0 μg. Indoleacetonitrile (IAN) shows a still lower activity and this relative lack of activity is also shown by most of the neutral auxins which have been found in various plant extracts. The high activity of IAN in the coleoptile cylinder test is now generally thought to be due to its partial conversion to IAA by an enzyme system present in the coleoptile and to subsequent synergism between these two compounds (11). It

would appear that this enzymatic conversion cannot occur to any appreciable extent in the *Coleus* petiole.

As far as is known the *Coleus* abscission test is specific for substances of the auxin type. It appears to be insensitive to most of the cell elongation inhibitors which occur in plant extracts, though, like all abscission phenomena, it is extremely sensitive to small amounts of ethylene. For this reason great care must be taken to ensure that the atmosphere of the laboratory where the test is carried out is free from all traces of coal gas or other sources of ethylene. It has been observed that certain anti-auxins which, in cell extension tests, inhibit auxin activity over a wide range of concentrations, may actually show synergistic effects with these same auxins in the *Coleus* test. An example of such synergism between NAA and trans-cinnamic acid is shown in Table I. Such synergism is particularly interesting in view of reports

Table I.

Synergism between trans-cinnamic acid and α-naphthaleneacetic acid (NAA) in the Coleus *abscission test.*

μg. trans-CA per petiole	Days' delay in abscission		
	No NAA	0·1 μg. NAA per petiole	0·3 μg. NAA per petiole
0	0	2·9	4·6
0·1	0	3·4	2·6
1·0	−0·1	4·9	6·5
10·0	0·1	6·6	11·5

that trans-cinnamic acid and other anti-auxins accelerate leaf abscission in cotton (4). This phenomenon is being studied further and will be reported in more detail in a later publication.

Factors influencing variability.

The variability of the test depends largely on the condition of the plants, but generally the means of 10 replicates agree to within 10 per cent. Where variability is greater than this, or where greater precision is required, 20 or 30 replicates may be used. Alternatively, since most of the variability is referable to the nodes, precision is greatly increased by making comparisons between the two opposing petioles at each node. In this arrangement one petiole of each nodal pair is treated with auxin and the other retained as an untreated control.

Of the experimental factors influencing variability, the length to which the petioles are trimmed is of importance. As shown in Table II, the effect of a given amount of auxin is less the greater the length of the petiole, so that, for greatest sensitivity and least variability, short petioles should be used and these should be cut to length as accurately as possible. 4 mm. is recommended as the shortest length it is convenient to use in practice. The cutting may be done on a cork block marked with guide lines.

Short periods of illumination during recording seem to have no effect on the rate of abscission. However, where petioles are left for several days in the light, ultimate abscission may be greatly delayed. Under these conditions the petiole takes over

TABLE II.

Effect of petiole length on the effectiveness of α-naphthaleneacetic acid in delaying abscission in the Coleus *test. Means of 20 petioles.*

Petiole length (mm.)	Mean number of days to abscission	
	Untreated	Treated NAA 0·25 μg. per petiole
2	0·5	5·8
6	0·5	5·5
10	0·5	4·4
14	0·5	3·6
18	0·5	3·3

the function of the missing lamina : chlorophyll develops and the delayed abscission probably indicates that auxin production has been initiated.

The time taken for the untreated control petioles to abscis varies with the season and with the condition of the plants. During spring and summer, at least 90 per cent. of the untreated petioles have usually dropped within 24 hours. In winter, or where the plants are low in vigour, two or three days may be required for abscission. At 15° to 20° C. abscission proceeds much more slowly than at 25° C. and accurate control of temperature to within $\pm \frac{1}{2}$° C. is essential in carrying out the test.

For the assay of auxin solutions of unknown concentration, a series of controls containing known amounts of a synthetic auxin is run with each test. NAA is the most convenient substance for use as a standard and a supply of discs containing 0·05, 0·1, 0·2, 0·4, 0·6 and 1·0 μg. may be prepared and stored for use as required.

Application to paper chromatography.

Owing to the fact that the auxin can be supplied directly to the test object on filter paper, without the need for elution, the *Coleus* test is particularly well adapted for the detection and measurement of auxins on paper chromatograms. By means of a paper punch, 0·5 cm. diameter discs are punched out along the length of the chromatogram and, after moistening in distilled water, each disc is placed on a *Coleus* petiole. Where a single chromatogram contains insufficient auxin to give a response in the test, it is possible to place two or more discs, punched from comparable positions on adjacent chromatograms, on a single petiole.

THE BEAN ROOTING TEST.

The ability of auxins to stimulate root formation on cuttings has frequently been used as the basis of test methods for the detection of these compounds. The test objects used have usually been woody or herbaceous stem cuttings, or petioles. Such tests are characterized by high variability (12), since the rooting response varies widely with the condition of the plant, the time of the year and the position from which the cutting was taken. Much of this variability can be avoided by using seedlings of peas or beans which, when separated from their root systems, will initiate

new root primordia on the hypocotyl or epicotyl in response to auxin treatment. Went (17) used the epicotyls of etiolated pea seedlings for a test of this type, but in darkness it is necessary to supply the seedlings with sucrose before they will respond to auxin applications. Under these conditions it is difficult to prevent fungal and bacterial contamination during the test. In the method described here green seedlings of *Phaseolus vulgaris*, variety Masterpiece or Red Kidney, are used, and the test is carried out in the light under ordinary glasshouse conditions.

Method.

The beans are sown in flats of sand in the glasshouse seven to nine days before they are required for the test. At least twice as many beans should be sown as are required, in order that a rigorous selection for uniformity may be made when the test is carried out. The temperature of the glasshouse should be maintained at 80° F. to encourage rapid and uniform germination. The seedlings are ready for use as soon as the first pair of simple leaves have emerged and the hypocotyledonary crook has straightened out. Uniform seedlings at this stage of development are selected, the cotyledons removed, and the hypocotyls cut to a length of 5 cm. They are then allowed to stand for an hour with the cut ends in water, shaded from direct sunlight. Any seedlings that wilt during this time are rejected.

The auxin is supplied to the seedlings in aqueous solution, a minimum of 5 ml. per 20 cuttings being required to carry out the test. 0·5 ml. portions of the test solution are placed in small glass vials (14×45 mm.) and two bean cuttings are stood in each tube. The cuttings are left in the shade until all the auxin solution has been taken up in the transpiration stream, a process which usually requires from two to four hours. They are then rinsed in water and immersed up to the cotyledonary node in jars of tap water. Jam jars (1 lb. or 2 lb.) with $\frac{1}{2}$-inch mesh wire netting across the top to support the cuttings are suitable. The jars, each with four or five cuttings, are finally stood in a large pan of water thermostatically maintained at 30° C. and shaded from direct sunlight. After 7 to 10 days the number of roots and root primordia visible on each hypocotyl is counted. Untreated control plants normally initiate between 30 and 40 roots during this period, and the number of roots on the treated seedlings is expressed as a percentage of the mean number on the control.

Concentration/response relationships.

With NAA, the number of roots produced is a linear function of the concentration over the range of 5 to 50 parts per million, and a similar relationship holds for IAA (Figs. 3 and 5). At concentrations below 5 parts per million the nature of the concentration/response relationship changes and becomes approximately logarithmic. As in the *Coleus* test, the neutral auxin IAN shows only little activity. The neutral fractions of certain plant extracts (e.g. of apple leaves), however, show high activity in the rooting test and presumably contain a neutral auxin other than IAN. The activity of certain other synthetic growth substances in this test is shown in Table III, which gives the concentration in parts per million required to induce the formation of twice as many roots as occurred on the untreated controls in the same test.

Variability.

Where the uniformity of the seedlings used for the test is high, the standard error of 20 replicates is of the order of 5 per cent. of the mean. Thus, differences between

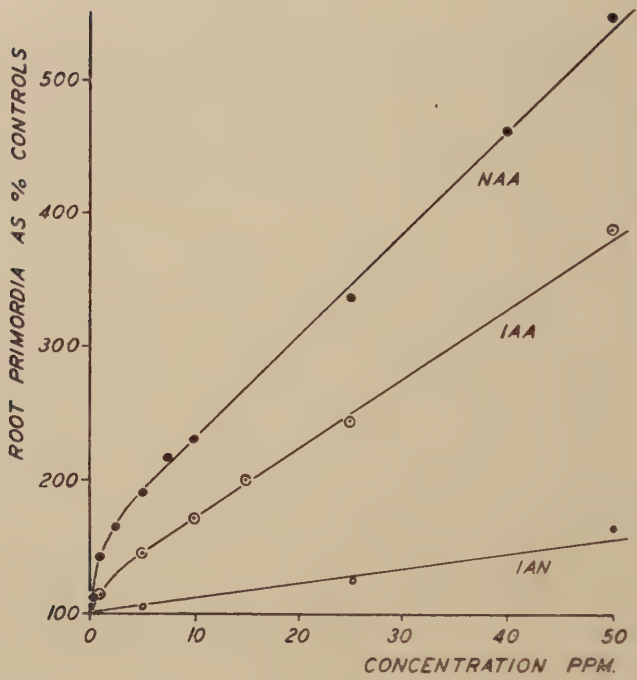

Fig. 3.

Bean rooting test. Relationship between concentration of growth substance and number of root primordia differentiated, for α-naphthaleneacetic acid (NAA), indoleacetic acid (IAA), and indoleacetonitrile (IAN).

Table III.

Activity of certain synthetic growth substances in the bean rooting test. The concentrations shown are those required for a 200 per cent. response.

Growth substance	Conc. p.p.m.	Remarks
α-naphthaleneacetic acid (NAA)	6	
α-(2,4-dichlorophenoxy) propionic acid	10	Lethal at 100 p.p.m.
Indoleacetic acid (IAA)	15	
Indolebutyric acid (IBA)	17	
Indoleacetonitrile (IAN)	90	Estimated by extrapolation.
2-naphthoxyacetic acid (2-NOA)	82	
Na 2-naphthoxyacetate	—	Inactive up to 100 p.p.m.
Na 3-chloro-2-naphthoxyacetate	—	Inactive up to 100 p.p.m.
2-3-5-tri-iodobenzoic acid	20	Abscission of epicotyls at concs. above 10 p.p.m.
Na-2-chlorophenylphthalamate	—	Inactive up to 100 p.p.m.
Na-3-methylphenylphthalamate	—	Inactive up to 100 p.p.m.

PLATE I

Fig. 4.
The *Coleus* abscission test, showing excised nodes arranged on strips of wet filter paper and treated with filter paper discs containing auxin.

Fig. 5.
Response of bean seedlings to α-naphthaleneacetic acid. *Left*, untreated; *centre*, treated NAA 5 p.p.m.; *right* treated NAA 50 p.p.m.

[*face p. 96*

treatments of the order of 14 per cent. are required for significance at the 5 per cent. level. By the use of 40 replicates the difference required for this level of significance may be lowered to 10 per cent.

Precautions in use of bean rooting test.

Hemberg (6) reported that, whereas hypocotyls of *Phaseolus vulgaris* rooted readily in tap water, they would not do so in distilled water, and he showed that this lack of response was attributable to lack of boron. Hence, in using the test, care should be taken to ensure that root formation is not limited by the boron content of the water used.

Unlike the *Coleus* test, the bean rooting test is not specific for auxins and a number of other compounds have been reported which produce responses of this type. Some of these, such as vitamin K and vitamin H′, increase rooting only in the presence of added auxin (7), and certain nitrogenous compounds can apparently act synergistically with the native auxin of the seedlings. Adenine is the most active of such compounds ; asparagine, guanine, choline and uracil are reported as having less effect (16). Certain mineral salts also influence the rooting response, particularly potassium nitrate which is stimulatory, and ammonium sulphate which, at concentrations as low as 2 parts per million, is strongly inhibitory (16). Hence, in using this test for the assay of naturally occurring auxins, it must first be established that the substance producing the response is actually an auxin and that the extract does not contain other substances likely to be active in promoting rooting. In using it for the assay of auxins which have been separated by paper chromatography, it is necessary first to elute the auxin from the paper. This can be done by irrigation of the paper with a small quantity of 90 per cent. alcohol. The eluate is then taken to dryness and the residue dissolved in the necessary quantity of distilled water for application to the bean seedlings.

SUMMARY.

1. Two methods for the bioassay of auxins are described. One of these is based on the ability of auxins to delay the abscission of de-bladed petioles of *Coleus blumei* ; the other on their ability to promote root formation on the hypocotyls of *Phaseolus vulgaris*.

2. In the abscission test, excised nodes of *Coleus* are maintained in a moist chamber at 25° C., and the auxin is applied to the cut ends of the petioles in small discs of filter paper. Within limits, the delay in abscission is proportional to the logarithm of the quantity of growth substance applied. This method is particularly convenient for the detection of auxins on paper chromatograms. It is believed to be specific for auxins.

3. For the bean rooting test, young seedlings of *Phaseolus vulgaris* grown in the light are used. They are severed 5 cm. below the cotyledonary node and allowed to take up a known amount of the test solution in the transpiration stream. They are then placed in jars of tap water to root. The number of roots and root primordia visible after 7 days is proportional, within limits, to the concentration of auxin in the solution.

4. The chief advantage of these tests is that they are relatively insensitive to the growth inhibitors which occur in extracts of plant tissues, and which frequently

interfere with auxin assays carried out by the more conventional methods based on cell extension response.

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THE RELATIVE IMPORTANCE OF STEM AND ROOT IN DETERMINING ROOTSTOCK INFLUENCE IN APPLES

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THE present work was designed to determine the parts played by the stem and root portions of apple rootstocks in controlling the vigour and fruiting of scion varieties. In 1930, when this experiment began, much was already known of certain aspects of rootstock influence, from the pioneer work of Hatton (1927, 1930) and others ; and a keen interest was awakening in the causes and exact seat of rootstock influence and in the reciprocal effects of scion and rootstock. A marked effect of the root system alone had been shown in young trees grafted direct on piece roots, and several workers had noted effects of rootstock stems, used as intermediates, on tree growth ; but trials specifically designed to compare the growth of mature trees grafted on rootstock stems with those direct on roots had not been carried out.

Swarbrick and Roberts (1927) and Roberts (1929, 1931, 1932) found a marked influence of the scion on the rootstock where the scions were grafted direct on pieces of one-year-old seedling root. The vigour of the scions in the nursery and the morphological characters of the root systems were fairly uniform in all seedling rootstocks grafted with the same scion variety. Scions did not show the same dominance over seedling root systems where the scion varieties were budded on the stem of the rootstock instead of being grafted direct on the root. From these observations on seedling rootstocks they drew the general conclusion that rootstock influence is situated in the stem rather than in the root and that, when the stem is eliminated by grafting direct on roots, the rootstock is dominated by the character of the scion. Vyvyan (1930) found that two-year-old root systems of M.II and M.VI retained their distinctive morphological features irrespective of the variety used as scion. He also showed that root systems of seedling stocks did not take on distinctive morphological characters according to the variety used as scion, whether or not a length of rootstock stem was left below the graft union. Beakbane (1941) found that rootstock differences in the anatomical structure of roots were present in both stem-grafted and root-grafted trees, and that the relative differences between the three clonal rootstocks tested were of the same order for both methods of working. There was no indication that the structure had been made more uniform by the influence of the scion, either in stem-grafted or in root-grafted trees. Hatton (1931) reported that rootstock influence was apparent in three- and four-year-old trees worked direct on clonal piece roots.

Between 1927 and 1954 several workers studied the effect of grafting pieces of stem of clonal rootstocks between seedling, or clonal, rootstocks and scion varieties. The use of intermediate stem-pieces to overcome incompatibility, or to attain greater uniformity of tree size in the nursery, had long been established practice, but the exact nature and mechanism of the influence of the stem-piece on the second scion had not been investigated. Grubb (1939*a*) found that intermediate stem-pieces had a

measurable effect on the growth and cropping of the second scion, but that the direction of the influence was by no means always indicated by the characteristics of the variety used as intermediate. He also noted an effect of length of intermediate, in that the influence on the vigour and the fruit characters of the varieties was more distinct where the intermediate was two feet long than where it was three inches. In another trial Grubb (1939*b*) showed that five-foot lengths of three vigorous apple varieties used as stem-builders, and allowed to bear leaves for a period, had an invigorating influence on the second scion in four-year-old trees. Root systems of M.IX were also invigorated in trees with long intermediate stem-pieces of these stembuilder varieties (Rogers, Beakbane and Field, 1939). Vyvyan (1938) demonstrated that, while intermediate stem-pieces of a dwarfing and a vigorous rootstock had a measurable effect, it was small compared with the influence of either type of root system. Swarbrick, Blair and Sham Singh (1946) studied trees of two apple varieties double-worked with intermediate stem-pieces of three clonal rootstocks. These trees showed that the presence of an intermediate stem of a dwarfing rootstock had a marked effect on tree size and fruit bud formation in trees up to eight years old. Similar size differences were noted at seventeen years old. Changes in the vigour of the second scion, induced by the use of various intermediate stem-pieces, were also reported by Müller and Borck (1953), Sax (1953), and Thomas (1954).

In the light of present knowledge several hypotheses could be advanced to explain the effect of intermediate stem-pieces :

(1) The speed of transport of water and metabolites may, to some extent, be controlled by the number and size of the elements of the conducting tissues ; it has been shown that a considerable range of variation occurs in the structure of these tissues in both rootstock and scion varieties (Beakbane and Renwick, 1936 ; Beakbane, 1941).

(2) Whatever the variety of intermediate used, some slight reduction of growth is likely to occur in double-worked trees owing to the presence of two unions. That a reduction in growth may be associated with the presence of a union has been shown by Vyvyan (1955).

(3) The metabolism of the intermediate stem-piece may differ from that of both top-scion and rootstock and may thus cause differences in the economy of the composite plant. Metabolic differences were noted between certain stocks, scions, and intermediates by Herrero (1951) for starch, and by Friedrich (1953) for catalase.

(4) It is possible that some of the results reported in the literature may have been due to the unwitting introduction of virus diseases with the intermediate. It has been shown that such diseases can cause a reduction in growth (Prentice, 1950 ; Posnette and Cropley, 1956).

Reviewing rootstock effects as a whole, Tukey and Brase (1933) concluded that no single part of a grafted tree could be considered to dominate the rest, but that all had been shown to have a marked influence on growth. They emphasized that the problem was rather one of relation and degree, the rootstock, the union, the top scion or the intermediate being the limiting factor according to circumstances. They stated, however, that in general the rootstock influence was the greatest. This last conclusion is in line with the work of Vyvyan (1955), who states that the rootstock has far more influence than the scion in determining tree weight.

The literature thus shows that, while much work has been done on various aspects of the parts played by stem and root in determining rootstock influence, the

information so far available does not fully elucidate the problem. In addition almost all the previous work has been on young trees in nursery trials.

The present trial was designed to contribute to the solution of the problem by comparing root- and stem-grafted trees on both seedling and clonal rootstocks from the nursery up to maturity.

Two main conclusions have been drawn : firstly, that the root portion of clonal rootstocks is able to exert an influence characteristic of the clone, in the absence of any rootstock stem ; and secondly, that the presence of a piece of rootstock stem tends to intensify the rootstock effect.

Material and method of examination.

The experimental trees were of Lane's Prince Albert apple, grafted high and low on stems, and direct on roots, of the rootstocks M.IX, M.II, M.XII and a set of seedlings, i.e. 12 combinations. Nine replicates of each combination were planted. Previous experience with these methods of grafting had shown that, to produce trees of comparable vigour in the nursery, it was important to use roots and stems of similar diameter. Therefore one-year-old rooted stool shoots and two-year-old seedlings were used for the stem-grafted trees, and eight-inch lengths of root cut from stools and from two-year-old seedlings for the root-grafted trees ; all these stems and roots were from 8 to 10 mm. in diameter.

Half the rooted stool shoots were cut back to 5 in. and half to 10 in. before grafting ; they were planted with 3 in. of stem below ground, leaving 2 in. and 7 in. respectively of rootstock stem above ground. Since the new tissues formed by rootstock stem below ground are found to resemble those of root (Beakbane, 1941), the treatments will be described hereafter by the length of stem above ground, viz. 2 in. and 7 in. The seedlings were worked at 2 in. and 7 in. above the topmost roots.

The experiment was laid out in twenty-seven plots, each of four trees. The three methods of working were applied to the plots so as to form three 3×3 Latin squares. The rootstocks were allocated at random to the four trees in each plot.

The field trial was planted in January 1932, with one hundred and eight two-year-old feathered trees. In this trial the unions of the root-grafted trees were placed below ground level and a regular inspection was made at three-monthly intervals to ensure that no scion roots were allowed to develop. These precautions were taken because in the earlier nursery trials part of the root of the rootstock had been grown above ground and, at the conclusion of the trials, it was considered possible that the structure of the roots above ground might have been modified by the changed environment. This conjecture was later found to be correct (Beakbane, 1941). The great care exercised to ensure that no scion roots should be allowed to develop did not in fact prove to have been necessary, as no scion roots emerged on these trees during the nineteen-year period of the trial. The variety used, Lane's Prince Albert, does not appear to form roots on stems at all readily. Scion-rooting has never been recorded at the Station for this variety.

Soon after planting, the trees were cut back to between 24 and 27 inches above ground level. In order to induce early fruiting and to avoid interference with the natural habit of the trees, no further pruning was done.

During the dormant season vigour was recorded by measuring trunk girth, length of new shoots, and height and spread of branches. A measure of fruitfulness was obtained by counting the number of fruit buds and by counting and weighing the

apples per tree. At seven years old the roots of certain trees were excavated, mapped, graded and weighed.

Statistical analysis of results.

A logarithmic transformation was used for the analyses of girth and shoot growth. This has now been standard practice at East Malling for some years for records of tree size.

In the early years all nine blocks were included in the analyses ; later, owing to the excavation of certain specimens, only five blocks were used. There was substantial agreement between the results of the analyses during both periods.

As it was thought that the rootstock effect might be greater where the greatest length of rootstock stem was present, particular attention was given in some of the analyses to the interaction of the largest rootstock effect (M.IX *v.* M.XII) with the treatments differing most markedly in the amount of rootstock stem present (root-worked *v.* worked on 7 in. rootstock stem).

RESULTS.

This experiment, lasting nineteen years, has shown that relatively small modifications in either stem or root growth resulted from the presence of a piece of rootstock stem. All trees showed the typical influence of the rootstock on which they were worked, whether stem- or root-grafted. There was, however, an indication that the presence of a piece of rootstock stem may accentuate the rootstock effect.

Vigour.

The general appearance of trees at 6 years old, on M.IX and M.XII, is shown in Fig. 1, which demonstrates clearly the large differences between the rootstocks, whether stem- or root-worked, and the small differences between methods of working. Trees on M.II and on seedlings were intermediate between the extremes illustrated ; the detailed measurements are discussed below.

(i) *Trunk girth.* The trunk girth measurements at nineteen years old (Table I) show large and highly significant differences between the trees on different rootstocks, whether worked on roots or on stems. Thus Hatton's (1931) finding that typical rootstock effects can be produced by the roots alone, in the absence of any rootstock

Table I.

Girth of trunk at nineteen years old.

Method of working	Rootstock				Geometric mean
	M.IX	M.II	Seedling	M.XII	
	cm.	cm.	cm.	cm.	
On root ..	16·6	30·9	36·3	46·8	30·9
On stem at 2″	11·5	28·2	37·2	49·0	27·5
On stem at 7″	12·9	26·3	36·3	50·1	28·2

Methods of working did not differ at the level P=0·05.
Significant ratio for rootstocks with the same method of working=1·24 (P=0·05).

stem, is confirmed ; and the present data show that these effects continue in the mature tree. No significant effect of method of working is shown by this Table. However, there is a suggestion that the presence of a piece of rootstock stem has made the trees on M.IX smaller and those on M.XII larger. Such an effect might be expected if the rootstock stem has an influence of the same kind as that of the roots and if the natural vigour of the scion (Lane's) is greater than that of M.IX and less than that of M.XII. There is evidence, from anatomical studies of stems of this variety, that the vigour of Lane's Prince Albert lies between that of M.IX and M.XII and that it is in fact rather more vigorous than M.II (Beakbane, 1941 ; Thompson, 1952) ; thus it is much more vigorous than M.IX and a little less vigorous than M.XII. The structure of roots of Lane's Prince Albert grown from layered plants (unpublished data), and growth records from field trials in which Lane's, M.IX and M.XII, used as scions, can be compared at the same age and on the same rootstock, though not on the same plot, also support this conclusion.

A separate examination of the largest rootstock effect, compared with the treatments differing most markedly in amount of rootstock stem, was therefore made for log. girth, and showed a significant interaction at the 5 per cent. level, indicating that the dwarfing tendency of M.IX was less marked in the root-grafted trees than in those with 7 in. of rootstock stem.

A comparison of the *cross-sectional areas* of the trunks of the stem- and root-worked trees on M.IX and M.XII was made. When the combination Lane's on M.IX with 7 in. of rootstock stem was taken as 100, the relative sizes of the others were as follows :

Method of working	*M.IX*	*M.XII*	*Ratio M.XII : M.IX*
On root	166	1,320	8·0
On 7 in. stem	100	1,513	15·1

These results show that the presence of a piece of rootstock stem increased the rootstock influence, the stem-worked trees on the dwarfing M.IX making less growth, and those on the very vigorous M.XII making more growth, than the root-worked ones. The ratio between the trunk size of root-worked trees on M.IX and M.XII is 8·0 and between the stem-worked trees on the same rootstocks it is 15·1 ; thus the contrast between the trunk cross-sectional areas of these trees on these two rootstocks was nearly doubled by the presence of a seven-inch piece of rootstock stem.

In any given rootstock/scion combination the magnitude of the rootstock stem effect will naturally depend on the difference between the rootstock stem and the scion stem. Where the contrast in vigour is large, as it is between Lane's Prince Albert and M.IX, the effect is relatively large, but where the contrast is small, as between Lane's and M.XII, then the effect is small (cf. p. 100).

The stem- and root-grafted trees on seedling rootstocks did not differ from each other in vigour. But since the mean vigour of the seedling rootstocks was evidently about the same as that of the scion variety, it is not likely that any effect of the seedling stems would be observable.

(ii) *Shoot growth.* Shoot growth was recorded annually from the date of planting until 1938. No difference in total shoot growth was found between the methods of working (Table II) nor were there any interactions between rootstock and method. Clear rootstock differences occurred in both root- and stem-worked trees.

TABLE II.

Total shoot growth (metres) per tree for the first nine years.

Method of working	Rootstock				Geometric mean
	M.IX	M.II	Seedling	M.XII	
	m.	m.	m.	m.	
On root ..	32	63	96	164	75
On stem at 2″	21	53	116	184	70
On stem at 7″	20	46	90	155	60

Methods of working did not differ at the level P=0·05.
Significant ratio for rootstocks with the same method of working=1·69 (P=0·05).

(iii) *Height and spread of tree.* At nine years old, the mean height of the trees from ground level and the spread of the branches, measured in two directions at right angles, were remarkably constant for all three methods of working. The mean heights were as follows : root-worked trees, 2·0 m. ; with 2 in. rootstock stem, 1·9 m. ; with 7 in. rootstock stem, 1·9 m.

Rootstock differences in branch spread occurred for all three methods of working (Table III).

TABLE III.

Spread of tree (metres) at nine years old.

Method of working	Rootstock				Mean
	M.IX	M.II	Seedling	M.XII	
	m.	m.	m.	m.	
On root ..	2·3	2·9	3·5	3·6	3·1
On stem at 2″	1·8	2·7	3·5	3·9	3·0
On stem at 7″	2·0	2·6	3·2	3·9	2·9

Methods of working did not differ at the level P=0·05.
Significant difference for rootstocks with the same method of working=0·5 (P=0·05).

Although the interaction did not reach significance at the 5 per cent. level, the relative tree sizes as measured by spread of branches showed that there was a tendency for the rootstock effect to be less with the root-grafted trees than with the trees worked on 7 in. of rootstock stem. This tendency is in agreement with the results for vigour shown by the analysis of girth.

FRUITING.

(i) *Fruit buds.* No effect of method of working was observed on the total number of fruit buds per tree, or on the terminal and axillary buds per metre of wood growth (Tables IV and V). During the early years both the stem- and root-worked trees on M.IX bore more fruit buds per tree than those on the more vigorous stocks, but at a later stage the number per tree was greater for the larger trees on the vigorous stocks (Table IV).

When the fruiting capacity was examined, as distinct from the total number of fruit buds per tree, it was found that both stem- and root-worked trees on M.IX

Table IV.

Mean number of fruit buds per tree.

Method of working	Third to sixth years					Seventh to tenth years				
	Rootstock					Rootstock				
	M.IX	M.II	Seedling	M.XII	Mean	M.IX	M.II	Seedling	M.XII	Mean
On root	74	64	32	13	46	369	555	743	814	620
On stem at 7″ ..	54	62	32	10	40	252	358	670	841	530

Table V.

Mean number of terminal and axillary fruit buds per metre of new growth, for the fourth to ninth years.

Method of working	Rootstock				Geometric mean
	M.IX	M.II	Seedling	M.XII	
On root ..	16·8	11·2	5·6	4·1	8·1
On stem at 2″	16·0	12·5	5·3	4·1	8·1
On stem at 7″	17·6	11·9	5·9	3·0	8·4

Methods of working did not differ at the level P=0·05.
Significant ratio for rootstocks with the same method of working=1·42 (P=0·05).

and M.II bore more terminal and axillary fruit buds per metre of wood growth than those on M.XII and seedling rootstocks during the whole period for which this record was made, i.e. until the ninth year. Thus, for all methods of working, the fruiting capacity of trees on the more precocious stocks remained greater than that of trees on rootstocks with a more delayed fruiting habit, even when they fell behind these trees in total production of fruit buds per tree owing to the disparity in tree size.

(ii) *Fruit.* No effect of the methods of working on total yield per tree was observed in the general analysis, except for one curious interaction (Table VI); the crop of the trees worked high on seedling stems was less than that of those worked

Table VI.

Total yield per tree.

Method of working	Rootstock				Mean
	M.IX	M.II	Seedling	M.XII	
	lb.	lb.	lb.	lb.	
On root ..	190	338	457	564	387
On stem at 2″	160	338	554	587	410
On stem at 7″	86	240	359	602	322

Significant difference for methods of working=65 (P=0·05).
Significant difference for rootstocks with the same method of working=141 (P=0·05).

low on seedling stems or direct on seedling roots. This result may be due to variation between individual seedling rootstocks in their influence on cropping capacity—a variation that has not been effaced by the influence of the scion variety (cf. also Beakbane, 1941, pp. 354 and 360). The interaction between the largest rootstock effect (M.IX *v.* M.XII), and the extreme difference in amount of rootstock stem (root-worked *v.* on 7 in. stem) did not reach significance at the 5 per cent. level, but showed a tendency for the rootstock effect to be greater for the stem-worked than for the root-worked trees.

Clear differences in yield occurred between the clonal rootstocks whatever the method of working.

Root systems of trees on M.IX.

During January and February 1937, trees on M.IX were excavated by the two methods described by Rogers and Vyvyan (1928 and 1934). The skeleton method was used for one root-grafted tree and one tree with 7 in. of rootstock stem above ground, and the block method for two root-grafted and two stem-grafted trees (worked at 7 in. above ground level). In the skeleton method the positions of the roots are marked on a plan as they are uncovered, thus making it possible to reconstruct the root system after excavation so as to show its original position in the soil. In the block method fresh and dry weights of root from soil blocks of known size and position are obtained.

The root systems of both stem- and root-grafted trees were typical of M.IX. In all six trees, shallow scaffold roots were present in the soil layer between 15 and 30 cm. below ground level. Vertical roots descended from this scaffold as far as the underlying rock. In general conformation, and in size of root-system relative to branch spread, the trees excavated by the skeleton method were similar to those of the same scion/rootstock combination previously excavated by Rogers and Vyvyan (1928). Fig. 2 shows that this typical root formation was present in the root-grafted tree.

The variation within treatments of the trees excavated by the block method was unusually high (Table VII). Even the stem/root ratio, which is normally fairly constant under similar nutritional conditions, varied from 1·49 to 2·40. The variation may have been due to the scattered distribution of the trees on the plot or to unequal competition from neighbouring trees of varying sizes in other trials ; but it was unrelated to the methods of grafting under investigation. Thus it may be concluded that the morphological characters of the root systems of M.IX were not influenced by the methods of working.

DISCUSSION.

The present trial has shown that, in the clonal rootstocks M.IX, M.II and M.XII, there is a powerful influence residing in the roots, quite independent of any influence in the stem. These rootstocks were able to produce their characteristic effect on the vegetative growth and fruiting of the scion, and to retain their typical habit of root growth, in the absence of any rootstock stem. The presence of the stem, however, also had an effect on growth, although this was small in comparison with the rootstock differences between trees grafted direct on piece roots.

It is not surprising that the *roots* should be capable of this degree of control over

Table VII.

Relative sizes of branch and root systems, and depth of rooting, in stem- and root-worked trees on M.IX.

Method of working	Tree number	At planting	At seven years old					
		Weight	Girth	Weight of head	Weight of root	Stem/root ratio	Amount of root less than 1 mm. in diameter	Amount of root deeper than 50 cm.
		gm.	cm.	gm.	gm.		%	%
On root	7·4	149	6·9	1,421	732	1·94	10·8	17·8
	9·7	113	7·9	2,023	1,362	1·49	7·8	18·9
	Mean	131	7·4	1,722	1,047	1·72	9·3	18·4
On stem at 7″	7·5	233	7·2	1,419	592	2·40	12·8	9·8
	8·1	219	6·8	1,136	668	1·70	12·4	3·7
	Mean	226	7·0	1,278	630	2·05	12·6	6·8

growth, as they commonly comprise one-third or more of the mass of the tree, excluding the leaves, and they normally absorb all the materials required for growth except the carbon and part of the oxygen. The effect of the *rootstock stem* in intensifying the rootstock influence may arise, at least partly, from the fact that in stem-grafted trees more of the total mass of the tree is composed of rootstock tissue. This would apply especially in the first few years. On the other hand, the rootstock stem may act in a manner comparable with intermediate stem-pieces, though not all of the mechanisms postulated for these on p. 100 would apply. In this instance there is only a single union, whereas with an intermediate stem-piece there are two ; and the risk of introduction of virus diseases, inherent in double-working methods, does not arise in comparing these stem- and root-worked plants of the same clones. Thus there remain the two possibilities of the stem effect being due to changes in the physical ease of transport in the conducting tissues, or to differences in metabolism between the rootstock stem, its own root, and the scion stem. In the first case, the length of the intermediate would be likely to influence the degree to which growth was affected and this has been found by Grubb (1939*a*) to be true for several series of trees ; in the second, presence or absence of stem might be the important factor rather than its length. In the present study no dramatic changes were associated with differences in length of stem, though it is true that these differences were not great, being two inches as against seven inches of rootstock stem above ground. It is indeed probable that often both the metabolic and the physical factors operate and that the balance between them varies.

Although these interesting effects of rootstock stems were found, the changes in growth were small compared with those associated with the root system ; and it is concluded that, in the main, the great effects of rootstocks on the growth and fruiting of the tree are due to the influence of the roots.

SUMMARY.

1. Lane's Prince Albert apple was grafted on M.IX, M.II, M.XII, and seedling rootstocks by each of three different methods, viz. on the rootstock stem at approximately 2 in. and 7 in. above ground level (with a 3-in. portion below ground) and direct on a piece of root of the rootstock. The trees were planted in a randomized field trial in 1932, and full records of vigour and cropping were maintained until the trees were nineteen years old.

2. The characteristic rootstock influence was found to be given by the roots themselves. It did not depend on the presence of a piece of rootstock stem. The presence of a piece of rootstock stem tended to enhance the rootstock effect, but the stem effect was much smaller than that of the roots. Some possible mechanisms of these effects, and of the effects of intermediate stems, are discussed.

3. At seven years old, the roots of certain trees were excavated, mapped, graded and weighed. No consistent differences were observed between excavated root systems of stem- and root-grafted trees. All were typical of the rootstock, whether a piece of rootstock stem was present or not.

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PLATE I

(a) Root-worked on M.IX.

(b) Stem-worked on M.IX.

(c) Root-worked on M.XII.

(d) Stem-worked on M.XII.

Fig. 1.

Six-year-old Lane's Prince Albert apple trees on M.IX and M.XII ; (a) and (c) bench-grafted on roots ; (b) and (d) grafted on the stem of the rootstock at 2 inches above ground level. Note large differences in vigour and amount of blossom between rootstocks in the absence of any rootstock stem, and small contrasts between methods of working.

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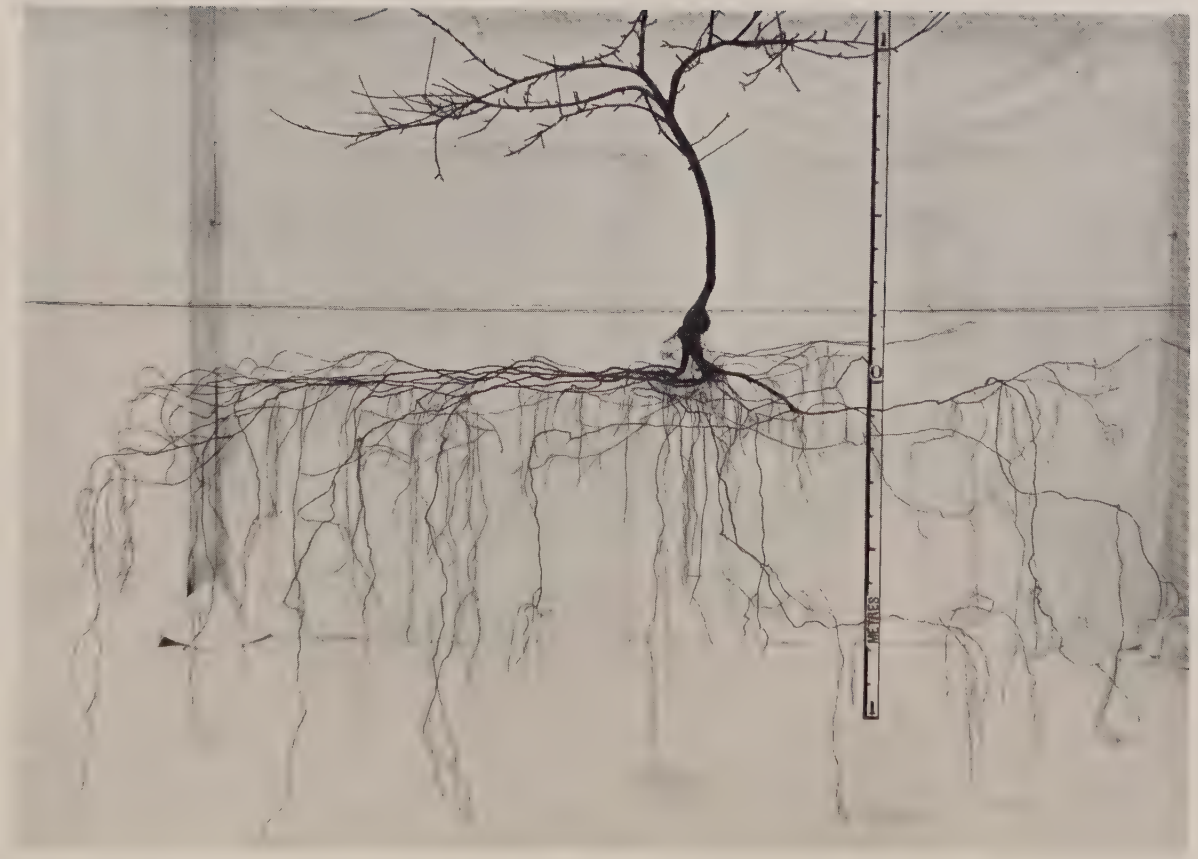

FIG. 2

A seven-year-old Lane's Prince Albert apple tree bench-grafted directly on a root of M.IX. Although no rootstock stem is present, the branches, trunk, bulge at union, and root-system are all typical of trees on M.IX

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STUDIES ON THE ETIOLOGY AND MODE OF SPREAD OF SCOTTISH RASPBERRY LEAF CURL DISEASE

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PRELIMINARY work on raspberry leaf curl (Cadman and Harris, 1952) showed that this virus disease was distinct from the mosaic diseases of raspberries. Unlike the latter, leaf curl was not transmitted by raspberry aphids though it spread rapidly in the field. It was apparently restricted to cultivated raspberries in eastern Scotland and killed the varieties Norfolk Giant, Malling Enterprise, Malling Jewel and Malling Notable ; others, such as Malling Promise, tolerated infection whereas Lloyd George and Malling Landmark seemed to be resistant. No insect vector was found nor were any alternative hosts of the virus discovered. A major economic problem was posed by the recurring and apparently spontaneous outbreaks of this devastating disease in commercial plantations.

Close scrutiny of recent leaf curl outbreaks in Angus and East Perthshire suggested that alternative hosts of the virus were plants other than *Rubus* species, and that raspberry plants became infected, either through their roots or through the young shoots when these broke through the soil surface. Experiments to test these hypotheses showed that leaf curl was caused by a number of differing ringspot viruses, mechanically transmissible from raspberry to other herbaceous plants including weeds and sugar beet ; and that sugar beet, and probably raspberry plants, became infected only when their roots were in contact with soil on which ringspot-infected plants had grown. The results are conveniently reviewed under the heads of mechanical transmission, the search for alternative host plants, and field experiments.

EXPERIMENTAL RESULTS.

MECHANICAL TRANSMISSION OF LEAF CURL VIRUSES.

There are few authentic records of mechanical transmission of viruses to or from Rosaceous plants and none concerning viruses that infect raspberry. Extracts of Rosaceous plants give strongly positive reactions to the customary tests for tannins, and raspberry and strawberry leaf extracts are known to contain substances that precipitate proteins and inhibit infection by viruses (Bennett, 1927 ; Bawden and Kleczkowski, 1945). For these reasons, difficulty in transmitting Rosaceous plant viruses mechanically has been widely attributed to the abundance of tannins in the leaves. In fact, little is known of the organic constituents of either raspberry or strawberry leaves and substances other than flavanols give the reactions often considered diagnostic of tannins (Bate-Smith and Swain, 1953). Alkaloids as well as proteins are precipitated by tannins, and Thung and van der Want (1951) found that nicotine sulphate protected the infectivity of tobacco mosaic virus when added to mixtures of raspberry leaves and infected tobacco leaves before grinding and pressing out the mixed saps.

Before 1953, all attempts to transmit leaf curl (Fig. 1) by rubbing sap from infected plants on leaves of healthy plants of raspberry, tobacco, cucumber and sugar beet had failed. Different results were obtained when nicotine sulphate was added to infected raspberry leaves before maceration. To 30 gm. infected raspberry leaves 4 ml. of 40 per cent. nicotine sulphate were added, the leaves ground in a mortar and the sap expressed through muslin. The dark brown precipitate that formed was separated from the opalescent orange-to-green supernatant by centrifuging for 10 minutes at 3,000 r.p.m. and the supernatant fluid dialysed overnight against running tap water.

The dialysate proved to be infective when rubbed on leaves of *Petunia*, *Nicotiana rustica*, *N. glutinosa*, *N. tabacum* var. White Burley, cucumber, *Physalis floridana* and *P. angulata* ; either necrotic or chlorotic lesions and rings developed on inoculated leaves (Fig. 2). The treated extracts of leaves and stems of diseased raspberries always contained ringspot viruses. A brown precipitate formed during dialysis and virus was present in both this and the supernatant fluid though not in the first precipitate thrown down by nicotine sulphate. No viruses transmissible to *Petunia* or tobacco were isolated from healthy raspberry seedlings, from raspberry plants free from leaf curl nor from those infected with veinbanding and other aphid-transmissible viruses.

During the winter of 1953/54 attempts were made to infect raspberry with the ringspot viruses isolated from leaf curl-infected plants, using sap from infected *Petunia* and *N. rustica*. Ultimately, 6 out of one batch of 400 Norfolk Giant raspberry seedlings inoculated in the 2-leaf stage showed symptoms resembling leaf curl some three weeks after inoculation. One of these developed unequivocal symptoms of leaf curl and a ringspot virus was subsequently isolated from it. The other 5 plants were not similarly checked. Raspberry plants seem to be extraordinarily difficult to infect by inoculating with infective sap but the authenticity of this one result is thought unquestionable.

Most of the leaf curl isolates caused necrotic or chlorotic lesions on inoculated leaves of *Datura stramonium*, *Hyoscyamus niger* and French bean (vars. Prince and Masterpiece), and when tests were made with these hosts it was obvious, from the variety of types of lesions developing on inoculated leaves, that infected raspberries often contained several viruses (Fig. 3). In 1954, samples from individual leaf curl infected raspberry plants were collected from some 12 different outbreaks in Angus and Perthshire, the viruses isolated from each and cultured in plants of *N. rustica*, *Petunia* and *Hyoscyamus*. Ringspot viruses were also isolated from diseased plants of Malling Exploit raspberry from S. Devon, sent by Mr. P. G. Allan, and of Malling Promise from Wiltshire, sent by Mr. F. A. Roach. The results of these tests were striking.

Sometimes one or several viruses were isolated from individual raspberry plants from the same plantation. An isolate from one plant from Blairgowrie caused lesions of 4 different kinds on *Datura stramonium* and cultures of virus from each kind of lesion caused distinctive symptoms on *Hyoscyamus*, *Datura* and *Nicotiana* spp. The viruses isolated from raspberry growing in different localities often differed widely and those from the Devon and Wiltshire samples seemed to be quite different from any isolated from raspberry in Scotland. Local-lesion isolates were made from several cultures and cross-protection tests were attempted with *N. rustica* and White Burley tobacco but these all failed. The *Nicotiana* plants failed to become systemically

infected with the first virus and leaves gave as many lesions when inoculated with this as with the challenging virus. Similar tests with other tobacco species and with *Hyoscyamus niger* were no more successful.

Work on the *in vitro* properties of these viruses has only recently begun and there is as yet no evidence to show whether leaf curl is caused by a series of related or unrelated ringspot viruses. Infected plants of one and the same raspberry variety often differ much in appearance but there seems to be no correlation between intensity of symptoms and multiple virus infection. Such differences may possibly be due to infection with differing viruses.

The search for alternative hosts.

Despite much searching, no wild *Rubus* plants infected with leaf curl were ever found, and outbreaks in commercial raspberry plantations seemed to be unconnected either with the previous history of affected stocks or with proximity to other infected plantations. Weeds therefore seemed to be the most probable sources of infection. Glasshouse tests made during the winter of 1953/54 showed that chickweed (*Stellaria media*), plantains (*Plantago lanceolata* and *P. major*), and groundsel (*Senecio vulgaris*) were susceptible and showed symptoms when inoculated with ringspot viruses isolated from raspberries. During 1954 several leaf curl affected raspberry plantations were searched for weeds showing ringspot symptoms. In one locality near Montrose, viruses that caused ringspot symptoms on *Petunia* and *N. rustica* were isolated from 9 common weeds of cultivated ground : *Myosotis arvensis, Tussilago farfara, Polygonum convolvulus,* thistles (*Cirsium arvense* and *Carduus nutans*), *Senecio Jacobaea, S. vulgaris, Stellaria media* and *Heracleum sphondylium* (Fig. 4). In the same locality sugar beet plants showing unmistakable ringspot symptoms were found in a commercial crop adjacent to the infected raspberries. Viruses were readily transmitted from these plants to beet but only to tobacco after they had been cultured in *Chenopodium amaranticolor*. Ringspot infected thistles and bindweed were found in two other raspberry plantations in Perthshire, infected clover and potato in another, but in others no infected weeds were found. There was no consistent association of leaf curl in raspberries with abundance of ringspot viruses in weeds.

The role of these weed viruses in the leaf curl problem is still obscure. Many of the isolates from weeds caused more severe symptoms on *Petunia* and *N. rustica* than those from raspberry. All the raspberry and weed isolates infected sugar beet but those from weeds consistently caused the more severe symptoms. The viruses from infected sugar beet, on the other hand, caused symptoms on *N. rustica* and *Petunia* more like those caused by some of the raspberry isolates. But all attempts to infect raspberry seedlings with viruses from either beet or weeds have failed.

Field experiments.

Field spread of leaf curl.

Spread of leaf curl in raspberry plantations usually follows either of two patterns. Less commonly, 80–90 per cent. of plants in patches up to 2–3 acres in extent may become infected in a single season. This epidemic spread has only been seen in young plantations and it results from infection of plants during their first or second growing seasons. More commonly, the disease spreads mainly up and down the rows from small infection foci, and there is an annual twofold increase in numbers of

infected plants. A map illustrating this second type of spread was published by Cadman and Harris (1952) and, because the data suggested spread of virus from one raspberry plant to another, an insect vector was postulated.

Throughout the past ten years, many attempts have been made by the writer and his colleagues to transmit leaf curl from infected to healthy raspberry and other plants by means of insects. Tests were made with aphids, leafhoppers, froghoppers, psyllids, capsids, thrips, mites, fleabeetles and other beetles found on raspberry foliage or roots, but these all failed ; nor was spread of infection correlated with presence of any particular insect species. In 1951, field experiments to establish the time of spread and mode of transmission of leaf curl were begun. These were made at first with raspberry and later with sugar beet. Malling Jewel raspberry plants were used because this variety, unlike most others, often shows symptoms in the year of infection.

Time of spread of leaf curl.

In 1951, 1952 and 1953 batches of 10 raspberry plants in pots of sterilized soil were exposed alongside leaf curl infected Norfolk Giant stools for successive fortnightly periods in plantations at Dundee, Forfar and Lunan Bay respectively. The 1951 experiment ran from 31st July to 30th September, the other two from late May to early September. At Dundee 0/30, at Forfar 1/90 and at Lunan Bay 1/100 of these potted plants showed leaf curl the following year. The single infection at Forfar occurred in the first batch, exposed from 26th May to 10th June ; that at Lunan Bay in a batch exposed during the first fortnight in September.

In the same years and places, raspberry plants, raised in sterilized soil, were tipped out of their pots and planted alongside leaf curl infected stools. At Dundee in 1951, 40 plants were planted out on 31st July and lifted on 30th September ; 22 of these showed leaf curl in 1952, although none of the potted plants became infected. At Forfar in 1952, one of 40 plants planted out on 26th May developed leaf curl within 5 weeks ; three more showed leaf curl in 1953. Ten plants were set out at Lunan Bay in 1953 but none became infected ; however, the disease spread little in this plantation that year.

In 1953 leaf curl developed in a small nursery of Norfolk Giant near St. Cyrus, Kincardine, a site specially chosen for remoteness from other raspberries. On 17th July, 47 Malling Jewel canes were planted amongst the infected Norfolk Giant and left until October. In 1954, 23 showed leaf curl, and ringspot viruses were isolated from all of these but from none of the symptomless plants.

The St. Cyrus site was cleared of raspberries in the autumn of 1953, cultivated and planted in April 1954, with three rows of Malling Jewel canes, the rows running the full length of the plot, some 100 yards long. The new canes covered the site of the 1953 leaf curl outbreak and also a second area in which infected raspberry plants had been found in 1952 and many ringspot infected weeds collected in 1953. One plant showing leaf curl symptoms was found on 12th June, some 6 weeks after planting ; 4 more on 19th June, 2 on 3rd July and one on 23rd July. All the plants were numbered serially, lifted in September and grown on. Of the total 500 plants, 160 showed leaf curl in 1955. Most of these came from two areas in the plot, one of which coincided almost exactly with that of the 1953 outbreak.

Sugar beet was sown between the raspberry rows at St. Cyrus in April 1954. Germination was poor and the rows were very incomplete. However, plants showing

ringspot symptoms were first found on 3rd July and others later that month and in August. From 14 sample plants, ringspot viruses were mechanically transmitted to sugar beet and *Chenopodium amaranticolor* and from the latter to *Petunia* and tobacco. On the last two hosts the beet viruses caused symptoms similar to those caused by raspberry isolates. Another experiment with beet, referred to again below, was begun in October 1954 and terminated in March 1955. Sap from some of these seedlings caused lesions on inoculated *C. amaranticolor* leaves.

These experiments showed that raspberry and sugar beet became infected with ringspot viruses at almost any time during the growing season from May to September and that raspberry plants could become infected within 5–6 weeks of planting. The final experiment with sugar beet showed that infection could occur during the autumn and winter.

Mode of transmission of leaf curl.

In the 1951 experiment at Dundee referred to above, the 40 Malling Jewel canes planted on 31st July were divided into two lots ; one of these was planted alongside growing but infected Norfolk Giant stools, the other in a part of the plantation where all raspberries had been killed by leaf curl. In each lot 11/20 plants became infected. A further treatment had been superimposed. In each lot of 20, the basal few inches of the canes of 10 plants were coated with greasebanding ; the canes on the other 10 were left untreated. Almost half as many (8/20) banded as unbanded (14/20) plants became infected. The greasebanding treatment was tested again at St. Cyrus in 1953 when 24 of 47 plants were treated after planting. This time the treatment had no effect, for equal numbers of banded and unbanded plants became infected. The most striking feature of the Dundee experiment was the fact that more than 50 per cent. of the planted canes became infected whereas none of the potted plants did so (*vide* p. 114).

Barriers to insect movement were tested in 1952 at Forfar, where 40 Malling Jewel plants were planted near to infected Norfolk Giant stools and enclosed individually in cages of 6, 14, 24 or 100 mesh cloth. There was an equal number of uncaged controls. Some cages were interfered with during the season, some of the 100 mesh ones damaged and there was little spread of leaf curl in the plantation that season. However, 4 of the uncaged plants and one in each of the lots caged with 6 and 100 mesh cloth showed leaf curl in 1953.

It seemed possible, therefore, that raspberry plants became infected through their roots, and an experiment designed to test this was made at Lunan Bay in 1953. Little spread of leaf curl occurred there in that year and the experiment failed. It was repeated at St. Cyrus in July 1954, with sugar beet seedlings in place of raspberries. Ten batches of 20 beet seedlings, raised in sterilized soil, were planted out in sterilized potting compost contained in polythene bags buried in the ground. Ten other batches were planted in open ground. The plots were some 18 in. in diameter and, after planting, 5 of each set of 10 were covered individually with cages of 100 mesh nylon cloth. Unfortunately, the nylon bags were stolen some two weeks before the plants were inspected on 25th September. Some of the plants showed ringspot symptoms and the numbers were distributed among the treatments as follows :

	Tops exposed	*Tops covered*
Sterilized soil ..	0/84	0/95
Plot soil	5/97	7/88

The 12 infected seedlings were grown on and viruses that caused ringspot symptoms on beet and *Petunia* were isolated from 10 of them. None of the apparently healthy plants was tested, however. A second trial of these treatments was begun in October and the plants harvested in March. All the seedlings with foliage exposed were lost during the winter and those surviving in the covered plots were too few and weakly to test individually for virus content. The survivors in each plot were therefore bulked and the sap from them rubbed on leaves of *Chenopodium amaranticolor*. Plants in 2 of the 5 lots grown in plot soil must have been infected, for sap from each lot caused lesions on the test plants. No lesions developed on leaves rubbed with sap from the 5 lots of seedlings grown in sterilized soil.

In October also, 50 pots were filled with top soil from the St. Cyrus plot, sugar beet seeds sown in them and the pots placed in the glasshouse. By January the seedlings were well grown and many showed ringspot symptoms. The roots and tops of all were separately tested for virus by rubbing the sap on leaves of healthy beet seedlings. Of 237 seedlings tested, 101 were infected ; of these, viruses were isolated from both roots and tops of 52, from the roots of 34 and from the tops of 15 plants. All the isolates caused local lesions on the inoculated beet leaves and some caused severe systemic symptoms.

These experiments conclusively establish some form of soil transmission as the mode of spread of ringspot viruses in sugar beet. The evidence that raspberry ringspot viruses spread in the same fashion is less convincing, but the conclusion that they do so is probably justified. Strong circumstantial evidence is provided by the continued failure to find an aerial insect vector, by the rarity of infection of potted plants even though plunged in the ground, and by the frequent failure of leaf curl to spread from infected to contiguous healthy plantations. An aerial vector efficient enough to spread leaf curl at the rates observed could hardly have entirely evaded detection, nor would spread be restricted in such a peculiar manner.

Attempts to find whether mechanical transmission of these viruses to plant roots occurs in the soil have so far failed. When *Nicotiana rustica* seedlings were grown in sterilized soil mixed with macerated infected leaves of *N. rustica* plants or watered with infective sap, none became infected. Raspberry seedlings failed to become infected when their roots were rubbed with infective *Petunia* or tobacco sap and no virus was detected in water leachates from soil samples taken from a leaf curl infected raspberry plantation at Blairgowrie.

DISCUSSION.

There is now unequivocal evidence that Scottish leaf curl is etiologically distinct from the analogous American alpha and beta leaf curl diseases of raspberry described by Bennett (1930). The former is caused by viruses mechanically transmissible and probably soil-borne ; the latter by viruses that are presumably not mechanically transmissible but are transmitted by *Aphis rubiphila*. While the name leaf curl is best retained for the disease described in this and other papers (Harris, Bryce and Foister, 1943 ; Cadman and Harris, 1952) it should be qualified by the prefix Scottish and the causal virus(es) should be named raspberry ringspot virus(es). Until more is known of the properties and interrelations of the viruses isolated from raspberry, weeds and sugar beet, further differentiation seems uncalled for. Leaf curl is descriptive of the symptoms shown by Norfolk Giant but not of those shown by other raspberry

PLATE I

Fig. 1.
Leaf of Malling Jewel raspberry with Scottish leaf curl disease.

Fig. 2.
Leaf of *Nicotiana rustica* systemically infected with ringspot virus from raspberry.

Fig. 3.
Leaf of *Chenopodium amaranticolor* showing two kinds of local lesions after inoculation with nicotine-treated sap from leaf curl infected raspberry.

Fig. 4.
Leaf of *Nicotiana rustica* systemically infected with ringspot virus from naturally infected *Myosotis arvensis*.

[*face p. 116*

varieties, such as Malling Jewel, that are now more widely grown. Viruses causing Scottish leaf curl disease may be confined to Scotland, but there is now evidence that similar viruses are more widely distributed.

The mode of soil transmission and the relationship of the weed and sugar beet viruses to the raspberry viruses are still obscure. Mechanical transmission of ringspot viruses to raspberry is difficult to effect and many experiments were made at Dundee where diseased and healthy plants grew side by side without any spread of virus occurring. In infected plantations leaf curl often spreads along the rows. Pruning and other mechanical operations are unlikely means of spreading virus, but movement of soil during cultivation might assist spread if the viruses are soil-borne. Frequently, however, the disease seems actively to advance through a plantation. Although it now seems most unlikely that there is an aerial vector of the disease, it is possible that a soil-living vector is concerned.

The viruses causing leaf curl kill susceptible raspberry varieties but there is ample evidence that they are perpetuated in some way. In one instance, 11 years elapsed between the grubbing of an infected Norfolk Giant plantation and the planting of healthy Malling Jewel canes on the same ground; yet the plants developed leaf curl the year after planting. Glasshouse tests have shown that raspberry ringspot viruses infect weeds and sugar beet so that perpetuation in alternative hosts seems likely. However, most of the viruses isolated from naturally infected weeds and sugar beet differ in certain respects from those isolated from raspberry, and there is no evidence that they can cause leaf curl.

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SUMMARY.

Scottish raspberry leaf curl disease is shown to be etiologically distinct from the analogous American raspberry leaf curl diseases and to be caused by a number of differing viruses which are sap-transmissible from infected raspberry to a variety of plants including weeds and sugar beet. Similar viruses have been isolated from diseased raspberry plants from England, and all caused local lesions and systemic symptoms on *Hyoscyamus*, *Datura*, *Petunia*, French bean and *Nicotiana* spp. The viruses isolated from raspberry grown in different localities often differed widely. Similar viruses have been isolated from weeds and sugar beet growing near infected raspberries, but none of these has been shown to cause leaf curl.

Both raspberry and sugar beet plants became infected at all times of the growing season, raspberry plants sometimes within 5 weeks of planting. Experiments showed that sugar beet and probably raspberries became infected only when their roots were in contact with soil on which ringspot infected plants had grown. An aerial vector of these ringspot viruses seems improbable and a soil-living vector likely.

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APPLE MOSAIC VIRUSES

HOST REACTIONS AND STRAIN INTERFERENCE

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ALTHOUGH apple mosaic occurs in most countries where apples are grown commercially, it has received comparatively little attention, probably because the disease usually appears to cause little damage to the crop and rarely spreads in orchards. The investigations reported in this paper were started in 1950, partly because a virulent strain of the virus had been found in Kent, and partly because certain features suggested that it would be a suitable virus disease of a tree crop for investigating host and virus strain relationships. Among these features were the conspicuous symptoms which plants of many varieties develop in the year after inoculation ; the absence or scarcity of an insect vector, so that cages need not be used for experiments in outdoor nurseries ; the presence of distinct strains, and the preliminary evidence of mild strains protecting plants from the effects of virulent strains (Posnette and Cropley, 1952).

HOST REACTIONS TO DIFFERENT STRAINS.

The three strains used in the experiments to be described were those previously called the " severe mosaic ", the " mild vein-banding " and the " mild mosaic " strains. For ease of reference, they will be referred to as " virulent ", " intermediate " and " mild ". The symptoms they cause are similar—creamy white areas frequently associated with the leaf veins—but differ markedly in degree on most varieties of apple. Moreover, varieties differ in their sensitivity, so that a mild strain may cause more marked symptoms on variety A than those caused by a more virulent strain on variety B ; but the mild, intermediate and virulent strains invariably produced symptoms in that order of intensity on all the varieties tested. Symptoms described as " slight " consist of small chlorotic flecks on comparatively few leaves, and confined to those produced on spurs or on growth made in the spring. Symptoms described as " intense " comprise extensive vein-banding and large chlorotic areas which become necrotic in late summer (leaves formed during the summer show symptoms, but less conspicuously than spring leaves). Intense symptoms are usually followed by partial defoliation in late summer (Fig. 6). Reddish-brown streaks may occur on the bark of young shoots, and cream-coloured patches on the fruit, these blemishes being very conspicuous on the red-fruited variety Lord Lambourne.

Because they can be propagated so readily, apple rootstock clones provide the most convenient indicator plants. The sensitivity of several clones was tested by inoculation with these three virus strains, both to compare the virulence of the strains and to provide information on symptoms likely to be seen by inspectors of the National Agricultural Advisory Service, who are responsible for rootstock certification.

In Table I, the rootstocks are listed in order of their sensitivity, ranging from M.XXV, which showed clear symptoms even with the mild strain and severe leaf

chlorosis, necrosis and fall with the virulent strain, to M.XVI, which developed localized conspicuous but non-necrotic symptoms with the virulent, and only a few chlorotic flecks with the intermediate strain.

TABLE I.

Sensitivity of some apple rootstocks to apple mosaic virus.

Rootstock clone	Symptoms caused by mosaic strain		
	Virulent	Intermediate	Mild
M.XXV	Very intense	Intense	Slight
M.IX	Very intense	Intense	——
MM.104	Very intense	Intense	Slight
MM.105	Very intense	Intense	Very slight
MM.102	Intense	Intense	None
Crab C	——	Intense	——
M.VII	Intense	Conspicuous	——
M.XII	——	Conspicuous	——
M.I	Intense	Conspicuous	——
90*	Intense	Conspicuous	Very slight
411*	Intense	Conspicuous	Very slight
M.IV	Intense	Conspicuous	——
MM.109	Intense	Conspicuous	Very slight
MM.106	Intense	Slight	Very slight
96*	Intense	Slight	Very slight
MM.111	Conspicuous	Slight	None
M.II	Conspicuous	Slight	——
M.XVI	Conspicuous, localized	Slight, localized	——

* Malling-Merton stocks not given commercial designations.
——, Not tested.

Definitions.

Very intense : symptoms of virulent strain evenly distributed over tree, with marked leaf necrosis and defoliation.
Intense : symptoms on most shoots, with some necrosis and defoliation.
Conspicuous : clear symptoms without necrosis, usually confined to spur and basal leaves.
Slight : small chlorotic areas, without necrosis, on spur leaves only.
Very slight : very small flecks on spur leaves only.

Luckwill (1954) has reported on the symptom expression of some apple varieties to a strain of mosaic virus. In Table II, other varieties are listed in order of sensitivity to East Malling strains, account being taken of the degree of defoliation, the extent of chlorosis and necrosis on the leaf, and the distribution of symptoms on the tree. Symptoms of the virulent strain were localized near the inoculation point on Newton Wonder, Laxton's Fortune and Lane's Prince Albert ; but affected leaves of these varieties differed markedly in their symptoms, which were bright yellow on Newton Wonder and Laxton's Fortune, with necrosis on the former, and pale green on Lane's Prince Albert.

Attempted transmission by expressed sap.

In May 1951, an attempt was made to transmit apple mosaic virus by sap inoculation. Leaves with intense symptoms caused by the virulent strain were macerated with a pestle and mortar ; the sap was squeezed out and gently rubbed on

the young leaves of twenty apple seedlings* previously dusted with fine carborundum. No symptoms developed on these test plants.

The experiment was repeated in 1952, with the difference that the leaves were

Table II.

Sensitivity of some apple varieties to apple mosaic virus.

Apple variety	Symptoms caused by mosaic strain		
	Virulent	Intermediate	Mild
Allington Pippin	Very intense	——	Slight
Lord Lambourne ..	Very intense	Intense	Slight
Beauty of Bath, Miller's Seedling, October Pippin, Worcester Pearmain, Hormead Pearmain, Bramley's Seedling, Cox's Orange Pippin	Intense	Conspicuous	Very slight
Newton Wonder	Intense, localized	——	Very slight
Laxton's Fortune ..	Conspicuous	Slight	None
Lane's Prince Albert ..	Slight*	Slight*	Very slight*

*Pale green mosaic.
——, Not tested.
Definitions as in Table I.

macerated in a phosphate buffer (pH 7·8) and five tobacco seedlings (var. White Burley) were inoculated in addition to nine apple seedlings. The latter were kept under observation for two years and no symptoms were seen ; the tobacco seedlings were also unaffected.

Since these experiments were done, Yarwood (1953) has reported the transmission of a virus to tobacco (and also to bean, cowpea, cucumber and tomato) by rubbing with discs freshly cut from apple leaves with mosaic. Later (Yarwood, 1955), he failed to transmit other types of apple mosaic and suggested that the mechanically transmissible virus was tobacco streak virus. The virus was returned to apple by *Cuscuta subinclusa* and caused mosaic symptoms.

Effect on yield.

A field trial was started in 1950 with the dual object of assessing the effect of two strains of mosaic virus on the cropping of four apple varieties, and of investigating the extent and method of spread from tree to tree, should any occur. A plot of trees on M.IX and 23 years of age was used, containing the varieties Cox's Orange Pippin, Worcester Pearmain, Allington Pippin and Newton Wonder, planted so that each row contained one tree of each variety, in randomized positions. All four trees in each row were either inoculated (by grafting) with the virulent or with the mild strain, or left uninoculated as controls, the treatments being randomized. Nine trees of each variety were given each treatment in August 1950, when shields of bark infected with the appropriate strain were grafted on to four branches orientated

* Seedlings were used in preference to budded plants because the former should be more susceptible and less likely to be infected with latent strains of mosaic virus.

approximately north, south, east and west. Trees on which the grafts died were re-inoculated in March 1951.

Records were taken each year of the total weight of fruit produced by each tree, and an estimate of fruit size was obtained by weighing and counting a sample of 30 lb. from each tree. Trunk girth was measured annually at a height of 1 ft. above the ground (i.e. 9 in. above the graft union). In the statistical analysis of the results, the yields in 1953 and 1954 were corrected by covariance on both yield and girth in 1950-51.

Effect on crop weight.

Biennial or irregular cropping of the trees complicates the interpretation of the effect of the virus strains, particularly as all the trees of a variety were not in phase, i.e. were not cropping heavily in the same years. The graphs (Fig. 1) show a tendency for the effect of the virulent strain to increase with the duration of infection, as would be expected because the spread of the virus through the tree, judged from the development of symptoms, was slow. In the most sensitive varieties, Allington and Cox, symptoms were distributed generally by 1952, but they were still localized on the inoculated branches of the Newton Wonder trees in 1954. Tests for virus localization, in which Lord Lambourne indicator plants were inoculated with buds from symptomless branches of these Newton Wonder trees, showed that the virus was in fact still restricted to the branches inoculated in 1950.

When the yields for 1953 and 1954 were added together, the depression (about 30 per cent.) in the crop of Cox's by both virus strains, and that (about 40 per cent.) of the Allingtons by the virulent strain were statistically significant ($P=0{\cdot}05$). The effect on the cropping of Worcester (26 per cent.) and Newton was not significant. Symptoms on the Newtons were so sparse that any reduction of their crop would not have been expected ; but the difference between the Worcesters and the Cox's in the significance of the result may be spurious, probably because 1954, the year when the greatest effect would be expected, was an " off " year for Worcester and an " on " year for Cox.

Effect on fruit size.

A comparison of Fig. 1 and Table III shows the usual tendency of fruit to be smaller with a heavy than with a light crop. It is noticeable, however, that the crop

TABLE III.

Effect of apple mosaic on fruit size.

Variety	Infection	Weight (lb.) of 100 apples in year				
		1950	1951	1952	1953	1954
Cox's Orange Pippin	Virulent	16·70	25·30	22·70	23·40	17·20
	Mild	16·60	23·90	21·60	25·40	16·80
	Healthy	16·10	25·50	22·50	25·10	17·00
Allington Pippin ..	Virulent	20·07	24·03	22·30	27·65	18·43
	Mild	19·12	27·23	19·74	24·86	19·17
	Healthy	20·37	27·25	20·93	25·62	21·82

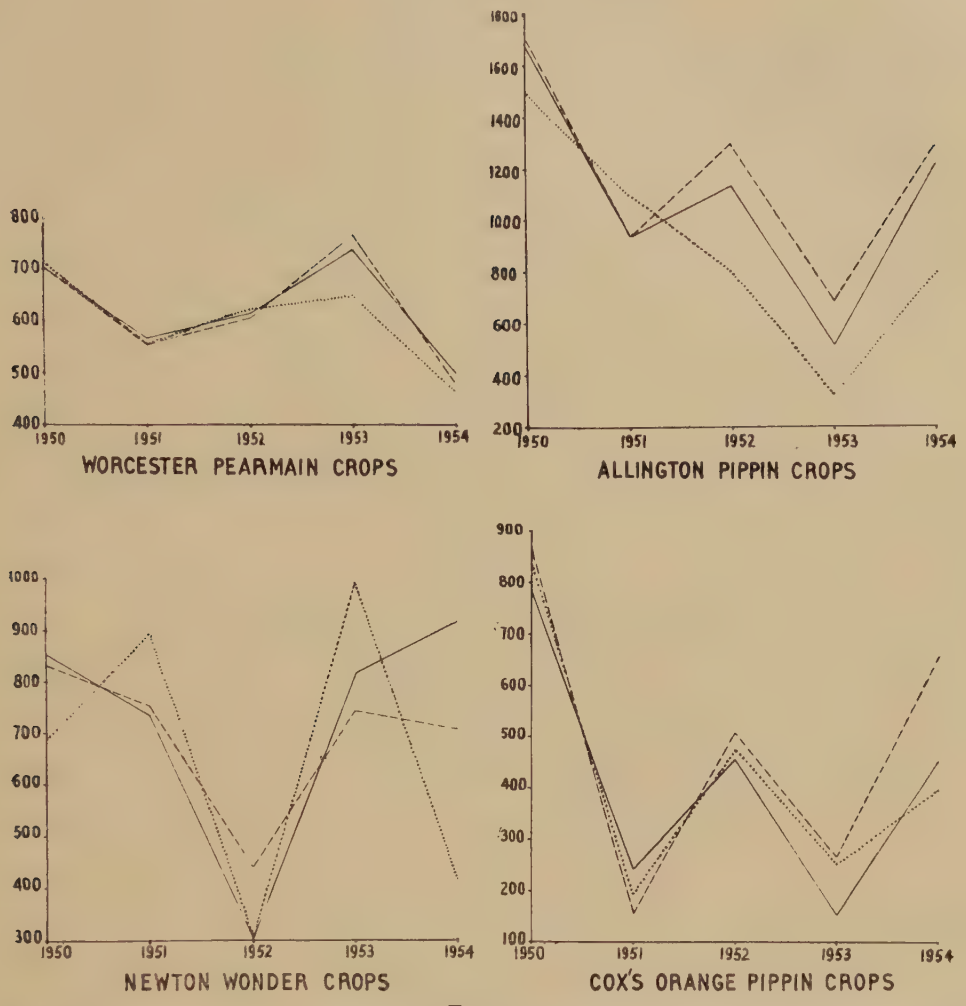


Fig. 1.

Effect of mosaic strains on cropping of four apple varieties in years 1950-54. Ordinates give total crop (lbs.) from nine trees.

Key to graphs: ..... trees infected with virulent mosaic strain.
——— trees infected with mild mosaic strain.
- - - - - - control trees.

reduction caused by apple mosaic was seldom accompanied by a marked increase in fruit size. In fact, the Allington trees infected with the mild strain produced a lighter crop of smaller apples than that on the healthy trees from 1952 onwards ; and although the apples on the trees infected with the virulent strain were larger in 1952 and 1953, they were smaller in 1954 than those on the healthy trees. There was little indication of any effect on fruit size in Cox's, except that the trees whose crop was reduced by the virulent strain should have produced larger fruit than did the healthy trees in 1954, but in fact their fruit was of the same size.

Effect on growth.

Two estimates have been made of the effect of apple mosaic on the growth of young Bramley's Seedling trees. In the experiment that first distinguished between strains of the virus, four trees had been inoculated in 1948 with the virulent strain, four with the intermediate and four were left uninoculated as controls. The trees were all 3 years old and comparable in size, but because they were inoculated in pairs and not randomized, the growth measurements cannot be treated statistically.

The stunting effect of the virulent strain on these trees was obvious to the eye 3 years after inoculation ; the latest measurements, taken in 1954, show differences between healthy and infected trees in height and girth of over 20 per cent. (Table IV).

Because trees usually become infected through the use of infected scions or rootstocks, the best estimate of the loss occurring in the field is obtained by comparing trees grown from healthy and infected budwood. An experiment on virus localization (described in the next section of this paper) resulted in two exactly comparable sets of trees, one comprising 14 infected, and the other 10 healthy trees, all propagated from the same infected " mother tree " on the same rootstock type (MM.102) on the same date. The measurements of these unpruned trees at the end of their second

Table IV.

Effect of apple mosaic on the growth of young Bramley's Seedling trees.

	Virus strain	Height (dcm.)	Girth (cm.)	No. branches (" feathers ")
Means of four trees on M.II, at 9 years old, and 6 years after inoculation	Virulent	18·7	17·8	
	Intermediate	23·7	21·7	
	Healthy	25·7	23·5	
2-year-old unpruned trees on MM.102 and propagated from same mother tree	Virulent	20·5	7·4	15
		13·5	5·5	7
		18·5	6·9	8
		19·0	7·0	7
		12·5	6·4	10
		16·0	7·2	13
		11·0	4·8	7
		21·0	7·7	17
		5·5	1·9	0
		19·5	8·5	13
		15·5	6·0	8
		18·0	7·2	10
		11·0	4·4	4
		12·5	4·3	1
		15·2	6·0	8·5 Mean
	Healthy	21·0	9·1	19
		21·5	8·8	16
		23·5	10·0	20
		22·5	9·2	17
		23·5	9·7	21
		22·5	8·6	14
		22·0	7·8	10
		23·0	9·5	16
		24·0	8·7	12
		22·6	9·0	16·1 Mean

year's growth are given individually in Table IV to illustrate the uniformity of the healthy set* compared with the extreme variation in the infected set. The mean height and girth show that the virus restricted growth by about 30 per cent., while " feathering " was partially suppressed.

Virus distribution and spread in orchards.

In the yield trial described above, no symptoms were seen on control trees despite their being adjacent to infected trees for 5 years. In other orchards under observation for 5 years, rare instances of apparent spread to trees adjacent to those with symptoms have been observed.

Most of the trees in the orchards on this Research Station have been indexed on the variety Lord Lambourne, which is sensitive to both mosaic and rubbery wood viruses (Posnette and Cropley, 1954). Of 3,845 trees indexed in 1952-3, 146 (3·8 per cent.) are infected with mosaic virus. Visible symptoms have been recorded in orchards indexed too recently for reliable data to be available at present. The distribution of infection is therefore known with considerable accuracy and, with the exception of four trees, the probable sources of infection were the scions, used either in the nursery or for top-working established trees. The history of these four exceptions is as follows :

1. Of 28 trees of Miller's Seedling frameworked on to Early Transparent in 1936, 13 are infected with mosaic ; but only 1 of 22 Miller's Seedling trees not frameworked is infected. It seems that the virus was either introduced with the Miller's graftwood or, more probably, was present in the Early Transparent trees, and the one ungrafted Miller's has since become infected by root grafting or some other agency.

2. A similar case occurs in another plot where Miller's Seedling was frameworked on to 56 Early Transparent trees in 1936 ; 32 of these are infected, but only 1 of 22 ungrafted Miller's trees.

3. In an orchard, planted in 1920, where 12 Bramley's Seedling trees were frameworked with Grenadier in 1939, 10 of these Grenadier trees are infected, but only one of the 61 unworked Bramley's.

4. In a 9-year-old orchard of 72 trees each of Jonathan, Ellison's Orange and Cox's Orange Pippin, 8 of the Jonathan trees were infected. One of the Ellison's was infected, possibly by virus spread from the Jonathans, but all of the Cox's were healthy.

Evidence of lack of spread is available from four other orchards where these last three varieties have been planted together, and the disease is confined to the Jonathan trees which were propagated from infected scion-wood.

Two 9-year-old orchards with alternate rows of Sunset and Laxton's Fortune trees provide further evidence. Over one-third of the Fortune trees are infected, but there has been no spread to the Sunset trees.

Latent infection.

Indexing 332 trees of Cox's, 281 of Jonathan, 262 of Ellison's and 54 of Laxton's Superb, with Lord Lambourne as test plants, has revealed four infected trees which have not shown symptoms during the period 1948 to 1955. Three of these are Cox's and one is Ellison's Orange ; the latter variety is known to be tolerant (Luckwill,

* Nine healthy trees were measured ; the tenth was broken accidentally.

loc. cit.) but, as Cox usually develops conspicuous symptoms, these latent infections require investigation before an explanation can be offered.

Among a further 1,950 trees indexed on Lord Lambourne, twelve trees on which symptoms had not been seen were found to be infected. These latent infections were in the varieties Crawley Beauty, Edward VII, Egremont Russet, Ellison's Orange, Laxton's Fortune, Grenadier, Jonathan, Laxton's Superb, Miller's Seedling and Orleans Reinette.

Experiments on virus localization.

In a previous paper by the present writers (Posnette and Cropley, 1952), the inconsistent transmission of the virulent strain was tentatively attributed to the virus not being fully systemic in the graft-wood used for inoculation. This hypothesis was tested in three ways : (1) by comparing transmission rates when scions with and scions without xylem were used for inoculation ; (2) by taking chips for inoculation in successive order from the base to the tip of young shoots ; and (3) by propagating from successive buds comparable with those used as inoculum in (2). In each experiment, shoots with and shoots without leaf symptoms, but which were otherwise comparable, were chosen for inoculum.

1. *The effect of including xylem in the inoculating scion.*

In July, 1952, five comparable pairs of budsticks, one with and one without leaf symptoms, were taken from a Bramley's Seedling tree which had been infected with the virulent strain since 1949 and on which symptoms were generally distributed. Two chips, one with and one without xylem, were cut from each of five internodes on each stick, and inserted in T-cuts in virus-free rootstocks, one to each stock. Thus there were fifty test plants inoculated with chips including xylem, fifty with chips without xylem and twenty-five uninoculated controls. All the chips united with the stocks and remained alive for 2 years.

All the test plants that had been inoculated with chips from symptom-bearing shoots developed intense symptoms in the following year, and clearly the virus had not been localized in the xylem tissues of the source plant.

The test plants were observed for 3 subsequent years and of those inoculated with chips from symptomless shoots, five " with xylem " and eight " without xylem " failed to develop symptoms.

2. *The effect of the age of the inoculating scion.*

In the experiment just described, the position of each inoculating chip on the graft-stick had been recorded. There were ten internodes on each stick and the chips were taken from five alternate internodes, starting from the one below the youngest expanding leaf. Of the thirteen test plants which failed to develop symptoms, ten had been inoculated with chips from the youngest internodes (first and third) ; two plants had received chips from the fifth and seventh internodes on a stick the younger internodes of which had also failed to transmit the virus.

3. *Virus localization and scion propagation.*

Two sticks of budwood, one with and one without leaf symptoms, were taken from each of two trees (Bramley's Seedling) which had been infected with the virulent strain for 5 years and showed well-distributed symptoms. The relative position of each bud was recorded as it was cut and budded on to a virus-free stock in August

1952. The stocks were cut back in the following spring and the scions grown for 3 years. Shoots from the rootstock were allowed to develop during 1953 so that leaf symptoms could be seen, but kept shortened to avoid undue competition with the scion.

Ten and fourteen stocks were budded respectively with buds from the two sticks with symptoms, and all developed symptoms of mosaic in the year after budding, as did all the scions except one which failed to grow. Ten stocks were budded successfully with buds from one of the sticks without leaf symptoms ; none showed symptoms after budding, and the ten scions have remained symptomless for 3 years. Seven stocks were budded successfully with buds from the other stick without symptoms ; the four youngest buds grew into symptomless plants, but the three oldest buds produced infected trees.

When one takes into consideration the virulence of the strain infecting this scion-wood, and the invariable expression of symptoms on the trees raised from budsticks with leaf symptoms, the assumption seems justified that the trees which showed no symptoms for 3 years were virus-free.

Heat inactivation.

Thomas (1937) and Louw (1944) reported unsuccessful attempts to inactivate apple mosaic virus by heating scion-wood in air or water at 36°, 48° and 50° C. We have used the lower temperature for longer periods with some success, following the method adopted for inactivating strawberry viruses (Posnette, Cropley and Ellenberger, 1953).

Apple seedlings were infected by chip budding with the virulent strain in July 1952 ; when showing severe symptoms 1 year later, fifteen were placed, still growing in their pots, in an incubator maintained at 37° C. They were removed in batches of 5 plants after 15, 27 and 35 days. Eight infected seedlings were kept in pots out-of-doors as controls.

On the day after their removal from the incubator, the plants were cut back and five buds from each were grafted on virus-free rootstocks, similar treatment being given to the controls. All the seedlings were planted in a nursery and symptoms recorded during the following two years.

Some of the heated plants (Table V) were symptomless during the year following heat treatment, but developed mosaic symptoms in 1955. The five plants treated for 27 days, and one of the plants treated for 35 days, have remained symptomless for two years, and sample budlings from these, which were retained for a second year, have shown no symptoms. By contrast, all eight controls developed clear symptoms, and so did nearly all their budlings.

None of the treated plants died and they did not appear to be damaged, although bud-take was poor after 35 days in the incubator. It would seem unnecessary in practice to treat plants for more than 27 days, a period with little effect on bud-take. The higher degree of virus inactivation obtained after 27 than after 35 days is probably fortuitous, and shows that the cure of all plants by a given treatment cannot be predicted.

Interference between strains.

Recent observations on the plants in the preliminary experiments (Posnette and Cropley, 1952) confirmed that the mild strain protected plants against the effects of

TABLE V.

Inactivation of mosaic virus in apple seedlings maintained at 37° C. in 1953.

Duration in days	Plant no.	Symptoms in		Budlings raised	No. with symptoms in 1954
		1954	1955		
15	1	—	+	5	4
	2	+	+	5	0
	3	+	+	5	2
	4	+	+	3	0
	5	+	+	5	1
27	6	—	—	4	0
	7	—	—	3	0
	8	—	—	3	0
	9	—	—	4	0
	10	—	—	5	0
35	11	+	+	0	—
	12	—	+	0	—
	13	—	+	1	0
	14	+	+	2	0
	15	—	—	1	0
Controls	16	+	+	5	5
	17	+	+	4	3
	18	+	+	3	3
	19	+	+	4	4
	20	+	+	5	5
	21	+	+	3	3
	22	+	+	5	5
	23	+	+	4	2

the intermediate strain for at least 4 years. This conclusion was supported by the results of a trial started in 1952 ; Lord Lambourne trees with and without the mild strain were propagated, and four healthy and four infected trees were inoculated by inserting bark chips containing the virulent strain, and four of each similarly inoculated with the intermediate strain. During the 2 subsequent years, no symptoms characteristic of infection with these strains developed on any trees previously infected with the mild strain (Table VI).

TABLE VI.

Protection by mild strain against virulent and intermediate strains.

Strain used for inoculation	Symptoms on Lord Lambourne	
	Infected with mild strain	Controls
Virulent	2/4 flecks only 2/4 grafts failed	3/4 severe 1/4 none
Intermediate	4/4 flecks only	2/4 vein bands 2/4 none
Not inoculated	2/2 flecks only	2/2 none

The ability of the intermediate strain to protect plants against the effects of the virulent strain was tested in an experiment using clonal rootstocks as test plants. Plants which had been infected with the intermediate strain for three years were either inoculated with the virulent strain, or left uninoculated as controls. Comparable healthy plants were inoculated similarly. All the buds used for inoculation united with the stocks and lived for the subsequent 2 years during which symptoms were recorded.

There were twenty-four plants in each group, four in each of the six clones M.I, M.II, M.IV, M.VII, M.IX and M.XVI. M.IV proved to be an unsuitable clone for this experiment because the symptoms caused by the two strains were not sufficiently distinct. Considering the twenty plants of the other clones, there was no difference in severity between the symptoms on the plants infected with the intermediate strain and those on the infected plants also inoculated with the virulent strain. Expressed numerically as 1 for slight, 2 for moderate and 3 for intense symptoms, the twenty plants which were healthy when inoculated with the virulent strain scored 44, those which were already infected with the intermediate strain when similarly inoculated scored 28, and those infected only with the intermediate strain scored 27.

Three new symptom types.

The symptoms caused by the three virus strains previously described and illustrated (Posnette and Cropley, 1952) differ chiefly in their intensity. Three other isolates with qualitatively distinctive leaf symptoms have since been studied ; we have no evidence on the relationship between these and the three strains of apple mosaic previously described, but descriptions are included here because they cause leaf symptoms and not, as far as is known, symptoms in the wood or on the fruit to link them with other known virus diseases of apples, viz. false sting, flat limb (Hockey, 1943), rubbery wood, chat fruit (Wallace, Swarbrick and Ogilvie, 1944), proliferation (Rui, 1950), rosette (van Katwijk, 1953) and rough skin (Mulder, 1955).

The *vein clearing type* was originally found in an M.II rootstock from a commercial nursery. The symptoms on M.II consist of a clearing of the fine veinlets with a distortion of some leaves as shown in Fig. 2. These symptoms developed on M.II stocks, near the point where bark-grafts were inserted, one year after inoculation. In successive years, the symptoms developed farther from the inoculation point, but even after 4 years they were not so generally distributed as on the source plant, which had presumably been raised from an infected stool.

Three plants of each of the rootstock clones M.I, M.IV, M.VII, M.IX and M.XVI were inoculated and did not develop symptoms in the subsequent 3 years ; Lord Lambourne scions grown for 2 years on infected rootstocks also remained symptomless. M.II is thus the only clone known to show symptoms of this strain.

Symptoms caused by the *line pattern type* were first observed in Laxton's Superb as a faint yellow wavy pattern. A range of rootstock varieties was inoculated and, with the exception of MM.102, all those that developed leaf symptoms showed a pattern of light green and pale yellow lines (Fig. 3). These varieties were MM.104, MM.105, MM.109, MM.111, M.XXV and three clones not yet given MM. numbers but known by their clone numbers as 90, 96 and 411 ; MM.102 developed small yellow flecks. MM.106 was the only tested variety which did not show symptoms within two years of inoculation.

Symptoms of the *ring spot type* have been observed only on leaves formed in the spring, later growth on the same shoots being symptomless. Cleared veinlets, often in the pattern of faint chlorotic rings (Fig. 4), were observed on the basal leaves of M.XII rootstocks and of Lord Lambourne scions during their first season's growth on this stock. In a survey of some 7,000 young Lord Lambourne trees, symptoms were seen only on the first few leaves produced by the scion after grafting on to either M.XII rootstocks, clone 90, or M.II stocks which had been inoculated with scions from orchard trees in surveys for rubbery wood virus (Posnette and Cropley, 1954). The evidence at present suggests that a high proportion of M.XII stocks are infected with this almost latent virus.

Non-transmissible variegation.

A type of variegation resembling mosaic was found in the variety Edward VII. Five trees were propagated from affected scion-wood, and all developed with variegated leaves (Fig. 5). Five trees propagated from normal scion-wood with an intermediate graft of affected wood between stock and scion grew into normal trees with no foliar symptoms during 3 years' observations.

A chimera-like variegation on a plant of the rootstock M.XXV was graft-perpetuated, but not transmitted to Lord Lambourne ; and attempts to transmit a sectorial variegation from Ellison's Orange to rootstocks MM.104, MM.105, MM.106, MM.109, MM.111 and 411 were unsuccessful. Similar spontaneous variegations have been seen on Cox's Orange Pippin, Sunset (Fig. 5), M.IV, MM.104 and 411.

DISCUSSION.

Previous reports on apple mosaic have been inconclusive in several respects. Louw (1944) and Hockey (1943) concluded that the disease was causing damage in South Africa and Nova Scotia respectively, but had no data from which to estimate the extent of the losses. Luckwill and Crowdy (1950) suggested that trees severely infected with mosaic should be grubbed, partly because they, like Blodgett (1938), found evidence of natural spread in orchards.

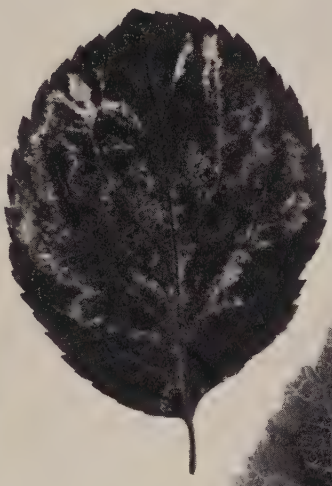
Our results have shown clearly that the virulent strain of apple mosaic depresses the crop of sensitive varieties, even when mature trees become infected. In our trial, the reduction of crop in the fourth year after infection amounted to 30 per cent. on Cox's Orange Pippin and Allington Pippin. The effect on the growth of young Bramley trees is such that much greater crop losses would be expected when trees are propagated from infected scion-wood. The evidence for crop loss caused by the mild strain is inconclusive, except for Cox's Orange Pippin. This strain is one of the mildest in the leaf symptoms caused by it that we have encountered ; most growers would be unaware of its existence in their trees and would certainly not consider it harmful. Our results to date suggest that such mild strains do reduce cropping, although their effect is exerted slowly. Future records on the yield trial, which is being continued, should enable us to assess crop losses more accurately.

We have not found evidence of spread from tree to tree, other than that which might be expected to occur occasionally by natural root grafting. In orchards where infection was present in trees of one variety when planted, there has been practically no spread to the neighbouring trees of other varieties during the life of the orchards

PLATE I

Fig. 2.
Vein clearing type in M.II rootstock (healthy leaf in centre).

Fig. 3.
Line pattern type in rootstock clone M.XXV.



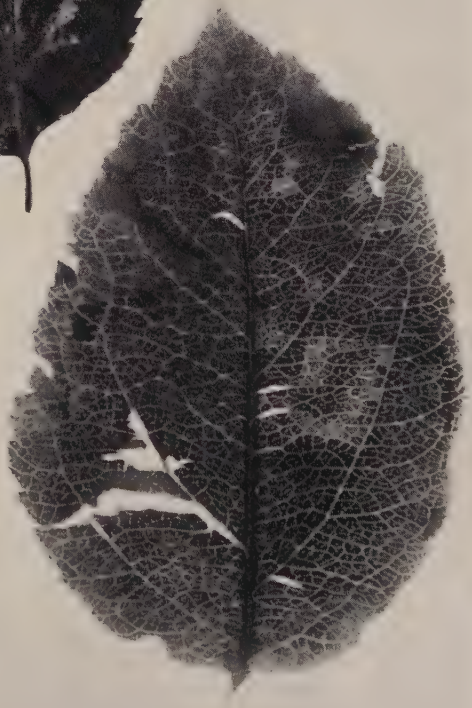


Fig. 4.
Ring spot type in Lord Lambourne.

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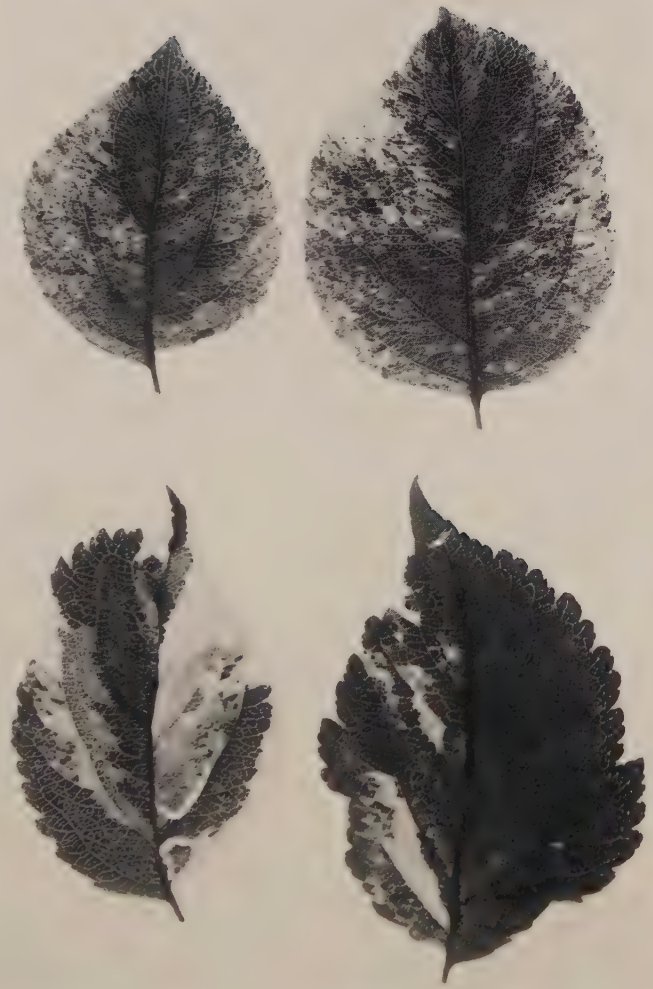

FIG. 5.
Non-transmissible variegation.
Above, Edward VII. *Below*, Sunset.

FIG. 6.
Miller's Seedling with: *left*, mild mosaic strain; *right*, virulent strain of mosaic virus.

(9 to over 30 years). We have, however, found several instances of latent infection, and Luckwill and Crowdy's statement that it is " always possible to say definitely whether the tree has mosaic or not " applies only to a sensitive variety like Lord Lambourne. One explanation of the apparent spread that other workers have reported is that trees with latent infection developed symptoms after the first survey.

Unlike Yarwood (1955), who was probably working with a different virus, we have failed to transmit apple mosaic virus by sap inoculation, using phosphate buffers and a fine abrasive on young leaves. Louw also failed with " mechanically contaminated " buds grafted to apple seedlings, and Hockey with sap injections. Transmission during pruning operations, as suggested by Blodgett, seems most improbable. While the occurrence of an insect vector cannot be discounted, particularly in New Zealand where the disease is most prevalent (Atkinson and Chamberlain, 1948), there seems no reason to doubt that spread is usually by grafting, either naturally between roots or during propagation.

When all available material of a variety is infected with apple mosaic, there are two ways by which virus-free stock may be obtained, that is, if the virus strain and apple variety behave like those in the present work—by propagation from young symptomless shoots, and by prolonged heat treatment of young trees growing in pots. Heating cut scion-wood is unlikely to inactivate the virus without damaging the scions irreparably. Chamberlain, Atkinson and Hunter (1953) concluded that the mosaic virus is systemic, because they obtained some infected trees when they propagated from symptomless shoots. Their figures show that they obtained 10 per cent. more symptomless trees when they used symptomless scions than when they used scions cut from shoots with symptoms ; our results suggest that some of their symptomless scions may have been virus-free. Luckwill (1954) also reported instances of apple mosaic virus being non-systemic in trees 25 and 48 years old.

Despite the failure of the virus to become fully systemic in the apple host, mild strains (causing only localized symptoms) afford a high degree of protection against the effects of more virulent (" challenging ") strains introduced by grafting into mature tissues. Presumably, any virus-free young tissues would not be thus protected, and the fact that they have apparently escaped invasion by virus translocated in xylem or phloem suggests that the challenging strain may be localized near the point of inoculation. What happens to a challenging strain inoculated into tissues protected by another strain is now being investigated.

SUMMARY.

Apple mosaic virus occurs in many different strains, and varieties differ markedly in reaction to them. 18 rootstock clones and 12 scion varieties were infected with two or more virus strains. Symptoms of a virulent strain varied from very intense on Lord Lambourne and Allington Pippin to slight on Lane's Prince Albert. M.IX and M.XXV were the most, and M.II and M.XVI the least sensitive of the stocks tested. Several of the rootstock clones showed no symptoms when infected with a mild strain.

In a field trial on mature trees, during the fourth and fifth years after inoculation, a virulent strain reduced the crop of Allington Pippin and Cox's Orange Pippin by 40 and 30 per cent. respectively, but had no significant effect on the crop of Worcester Pearmain and Newton Wonder.

The growth of young Bramley's Seedling trees in the nursery was reduced more than 20 per cent. by a virulent strain.

The virus was found to be non-systemic in rapidly-growing shoots, and healthy trees were propagated from buds taken from the tips of such shoots.

The virus was inactivated in plants maintained at 37° C. for 27 days. Some plants were permanently cured by this treatment ; from others not cured, healthy plants were propagated from buds removed immediately after treatment.

Plants already infected with a mild or with an intermediate strain developed no symptoms after inoculation with a virulent strain.

The symptoms of three " new types " of mosaic virus and a non-transmissible variegation are described.

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THE APPLICATION OF RAPID CHEMICAL TESTS TO THE DIAGNOSIS OF MINERAL DEFICIENCIES IN HORTICULTURAL CROPS

IV. A COMPARISON BETWEEN THE TOTAL NUTRIENTS AND THOSE EXTRACTED BY BUFFER SOLUTIONS FROM POTATO LEAVES

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It has been established that a diffusion method for extracting mineral nutrients from fresh plant tissues can be used in the field to diagnose mineral deficiencies in crop plants (1, 2). In the laboratory, maceraters, including Waring and NIRD blendors, have been used to extract mineral nutrients from fresh plant tissues, as the methods involving their use are quicker and more readily standardized than the diffusion method. With an acetate buffer (Morgan's reagent) as extractant, the results obtained by use of maceraters were found to be closely correlated with total amounts of the nutrients in the tissues (3, 4).

In Part III of this series an account was given of experiments made at Long Ashton in 1952 when fresh potato leaves were extracted with acetate and citrate buffers, and in 1953 when malonate and succinate buffers were used. It was then shown that the nutrients extracted by the acetate and citrate buffers were better correlated with the total amounts in the tissues than were those extracted by malonate or succinate. In the present paper, data discussed in that paper are examined in relation to manurial treatments, seasonal trends and crop yields.

Materials and methods.

Full details of the manurial treatments have already been given (4). The following six treatments were replicated in each of three blocks : *nil*, farmyard manure (FYM), NPK, NK, NP and PK.

The following samplings of potato leaves were made :

1952.	Kerr's Pink ..	24th June, 14th July and 21st August.
	Dunbar Standard	25th June, 15th July and 22nd August.
1953.	Kerr's Pink ..	18th June and 15th July.

Mid-stem leaves of potato were collected from each plot ; sub-samples were extracted with buffered solutions and others were dried for the determination of the total nutrient content, as described previously (4). Chemical determinations included K, Mg, Ca, nitrate N and total N ; P was determined in the 1953 samples only.

RESULTS.

1952 season.

The values obtained for K, Mg, Ca and nitrate N in acetate and citrate extracts of leaves of Kerr's Pink, for each manurial treatment, are compared with the total leaf contents in Table Ia. The values for Dunbar Standard are given in Table Ib.

Table Ia.

Nutrient content of Kerr's Pink leaves (1952) as determined by acetate and citrate extraction methods and by total analysis.

Treatment	Potassium			Magnesium			Calcium			Nitrogen			Mean yield (ware) lb. per plot
	Method			Method			Method			Method			
	Total	Extraction		Total	Extraction		Total	Extraction		Total	Extraction		
		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate	
Nil	1·59	1,459	1,183	0·79	1,088	573	4·28	4,710	4,598	4·4	707	420	75
FYM	3·20	2,471	2,051	0·74	986	573	4·11	4,065	4,065	4·0	368	267	228
NPK	3·16	2,990	2,517	0·50	868	381	4·11	4,376	3,884	4·1	440	326	216
PK	3·40	2,770	2,536	0·37	739	356	3·84	3,928	3,917	4·1	414	290	183
NK	3·42	2,919	3,026	0·55	783	467	3·14	3,257	3,165	4·7	715	572	173
NP	1·25	983	915	0·74	1,019	555	4·73	5,390	4,846	5·0	893	536	51
*S.E.**	*0·192*	*172·4*	*172·4*	*0·075*	*47·3*	*47·3*	*0·22*	*369·7*	*369·7*	*0·24*	*58·9*	*58·9*	*29*
S.E.†		*150·9*			*45·9*			*245·9*			*55·2*		
Mean	2·50	2,286	2,038	0·61	914	474	4·03	4,288	4,079	4·4	590	402	154
S.E.‡		*61·6*			*18·7*			*317·5*			*22·6*		

* S.E. of differences between individual treatment means.
† S.E. of differences between method means for acetate and citrate extracts. S.E. $\times 2$ is approximately $P<0·05$.
‡ S.E. of differences between general means for acetate and citrate extracts. S.E. $\times 3$ is approximately $P<0·01$.
Total : % K_2O, MgO, CaO and N in dry weight.
Acetate and citrate extracts : μg. K, Mg, Ca and nitrate N, extracted per gm. fresh weight.

Table Ib.

Nutrient content of Dunbar Standard leaves (1952) as determined by acetate and citrate extraction methods and by total analysis.

Treatment	Potassium			Magnesium			Calcium			Nitrogen			Mean yield (ware) lb. per plot
	Method			Method			Method			Method			
	Total	Extraction		Total	Extraction		Total	Extraction		Total	Extraction		
		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate	
Nil	2·09	1,549	1,586	0·84	1,242	456	4·11	6,192	4,569	4·6	1,008	890	75
FYM	4·59	3,212	2,953	0·75	1,030	347	3·48	4,999	4,583	4·4	444	503	261
NPK	4·33	3,508	3,262	0·56	960	246	3·93	5,444	4,113	4·4	411	488	255
PK	4·84	3,350	3,536	0·57	949	84	3·92	5,392	4,321	4·5	571	707	205
NK	4·81	3,317	3,652	0·65	978	338	3·57	4,955	4,291	4·7	832	1,013	182
NP	1·63	1,096	1,438	0·72	1,099	377	4·02	6,851	4,813	5·0	1,145	1,185	82
*S.E.**	*0·135*	*82·5*	*82·5*	*0·037*	*52·0*	*52·0*	*0·25*	*439·2*	*439·2*	*0·2*	*96·4*	*96·4*	*25*
S.E.†		*303·2*			*41·4*			*549·5*			*79·4*		
Mean	3·71	2,505	2,738	0·68	1,043	308	3·84	5,638	4,448	4·6	748	797	176
S.E.‡		*123·8*			*53·3*			*224·3*			*32·4*		

Notes as in Table Ia.

All values are the means for three blocks and for the three dates of sampling. The data are illustrated in Fig. 1.

Table II gives the mean values for the three blocks and the six manurial treatments determined for each date of sampling and shows the seasonal trend in nutrient status.

Potassium deficiency was the main factor depressing yields of both varieties; the yield from the NP plots was not significantly different from that of the *nil* plots (Tables Ia and Ib). The deficiency was clearly shown by all three methods of analysis, the values in the acetate and citrate extracts being of the same order. There was a marked fall in extractable potassium as the season advanced.

All methods of analysis showed lower *magnesium* contents in plants receiving potassium; the levels increased throughout the season.

The *calcium* level was greatest in plots not receiving potassium and, as with magnesium, the values were highest at the end of the season, whether determined in extracts or as the total amounts in the leaves.

At the first sampling date the level of *nitrate nitrogen* in Kerr's Pink was significantly lower in extracts of plants from the PK and FYM treatments than in those given NPK. This difference was not, however, shown by the total N contents of the leaves nor by the citrate and acetate extracts at the second and third samplings. Previous work (1, 4) has shown that the nitrate N content of the acetate extracts is not a reliable index of N deficiency late in the season.

At all three sampling dates a significant increase in nitrogen status in plants given N treatments was shown by all methods of analysis. The levels of total N and of nitrate N were lowest in the later samples.

Tables Ia and Ib show that acetate extracts contained about twice to three times as much Mg (for both potato varieties), significantly more Ca (for Dunbar Standard only) and significantly more nitrate nitrogen (for Kerr's Pink only) than did the citrate extracts.

1953 SEASON.

Only Kerr's Pink was grown in 1953, when malonate and succinate buffers were used to find whether these extracts gave a better indication of crop performance than had been recorded by the acetate and citrate buffers in 1952. Values obtained for K, Mg, Ca, nitrate N and P for each manurial treatment, compared with total amounts present in the leaves, are shown in Table III, which also gives the mean yield for each treatment. The nutrient data are means for three blocks and three dates of sampling.

Potassium deficiency was again the main factor influencing yields and all methods of analysis reflected this deficiency clearly. The K status of the leaves decreased as the season progressed, thus confirming the data obtained in 1952.

Both malonate and succinate extracts showed reduced *magnesium* and *calcium* levels in plants given potassium; for Ca this reduction was much more apparent than in values for total Ca content. All methods showed that Ca increased with the age of the plant. For magnesium, however, while there was a steady increase in the total content throughout the season, there was a decline in the amount extracted by both malonate and succinate. The correlation between the total and extracted magnesium was therefore poorer than that obtained with acetate and citrate buffers in 1952.

The *nitrate N* level in both extracts was significantly lower for the PK treatment

Table II.

Mean values of nutrient contents for three blocks and six manurial treatments at each date of sampling.

Sampling dates	Potassium			Magnesium			Calcium			Nitrogen		
	Method			Method			Method			Method		
	Total	Extraction		Total	Extraction		Total	Extraction		Total	Extraction	
		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate		Acetate	Citrate
Kerr's Pink												
24th June, 1952 ..	2·22	3,263	2,735	0·44	539	157	3·11	3,562	2,755	4·80	646	662
14th July, 1952	2·16	1,946	1,635	0·54	1,045	635	4·04	3,785	3,764	4·60	660	197
21st August, 1952 ..	2·03	1,590	1,744	0·86	1,158	631	4·80	5,518	5,720	3·80	463	346
*S.E.**	*0·08*	*150*		*0·04*	*36*		*0·12*	*190*		*0·13*	*55*	
Dunbar Standard												
25th June, 1952 ..	5·83	2,924	3,401	0·46	839	235	2·91	5,807	4,109	4·90	850	1,117
15th July, 1952 ..	3·11	2,624	2,625	0·57	1,267	206	3·92	4,766	3,066	4·80	831	823
22nd August, 1952 ..	2·20	2,469	2,186	1·01	1,024	529	4·69	6,343	6,170	4·10	525	453
*S.E.**	*0·11*	*204*		*0·04*	*36*		*0·23*	*441*		*0·12*	*79*	

* S.E. of differences between sampling means for acetate and citrate extracts. S.E. $\times 2$ is approximately $P<0{\cdot}05$. S.E. $\times 3$ is approximately $P<0{\cdot}01$.

Total : % K_2O, MgO, CaO and N in dry weight.

Acetate and citrate extracts : μg. K, Mg, Ca and nitrate N extracted per gm. fresh weight.

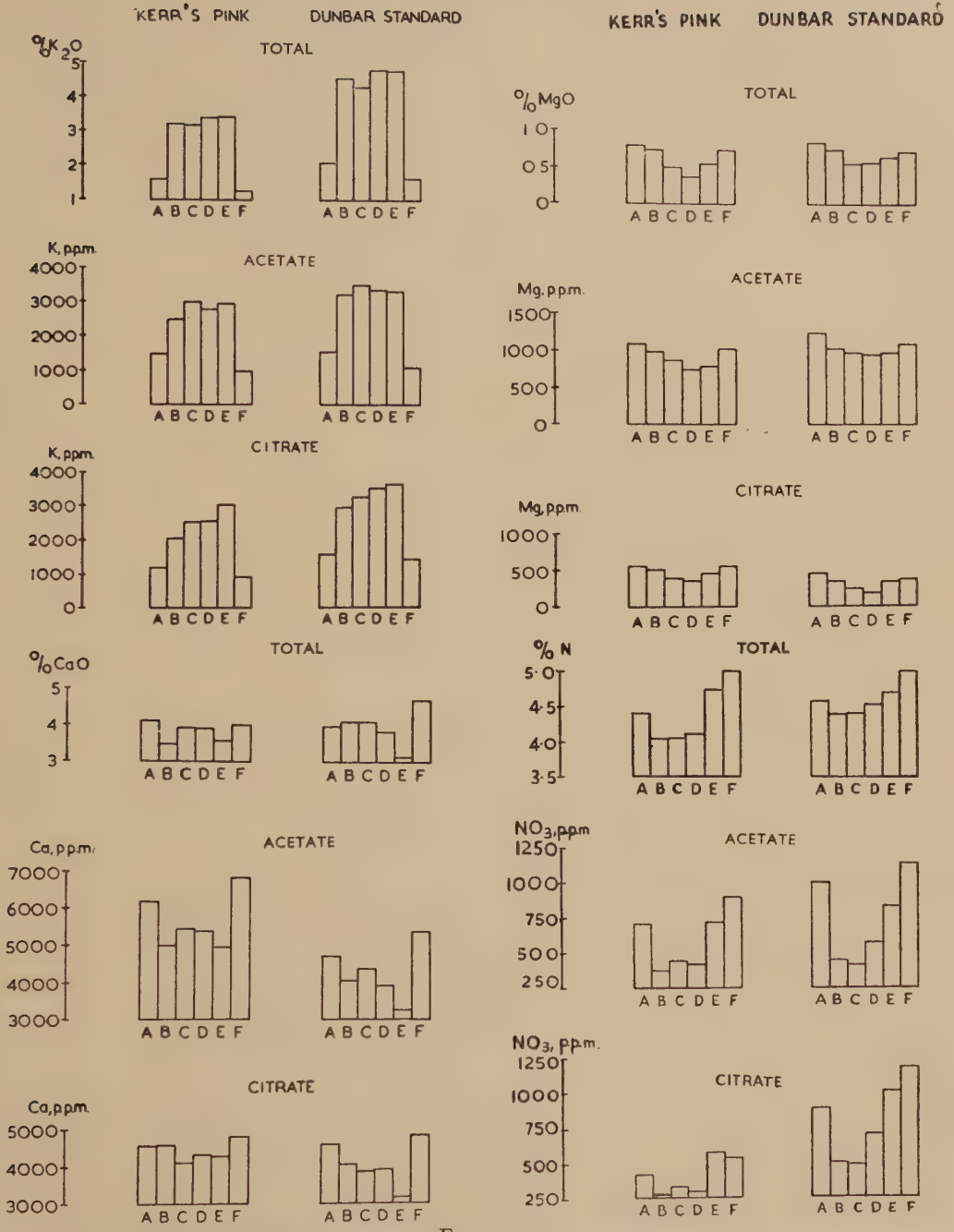

FIG. 1.

Comparison of total contents of K, Mg, Ca and N in potato leaves (1952), vars., Kerr's Pink and Dunbar Standard, with the amounts extracted in acetate and citrate buffers.
Manurial treatments (ordinates): A, *nil*; B, farmyard manure; C, NPK; D, PK; E, NK; F, NP.

Table III.

Nutrient content of Kerr's Pink leaves (1953) as determined by malonate and succinate extraction methods and by total analysis.

Treatment	Potassium			Magnesium			Calcium			Nitrogen			Phosphorus			Mean yield (ware) lb. per plot
	Method			Method			Method			Method			Method			
	Total	Extraction		Total	Extraction		Total	Extraction		Total	Extraction		Total	Extraction		
		Malonate	Succinate		Malonate	Succinate		Malonate	Succinate		Malonate	Succinate		Malonate	Succinate	
Nil	1·97	1,521	1,404	0·72	426	443	5·05	3,394	3,568	4·16	603	686	0·57	105	115	94
FYM	3·20	2,520	2,542	0·46	396	353	4·76	2,770	2,265	4·02	591	724	0·55	61	85	182
NPK	3·37	2,686	2,536	0·30	291	285	4·67	2,636	2,420	4·46	855	913	0·54	92	95	262
PK	3·51	2,586	2,697	0·44	231	247	4·66	2,416	2,355	4·04	466	520	0·58	66	88	137
NK	3·88	2,642	3,041	0·50	361	347	4·01	2,571	2,516	5·14	969	791	0·60	90	80	157
NP	1·35	1,260	1,277	0·73	421	397	5·07	3,813	3,530	5·30	875	891	0·74	135	154	91
*S.E.**	*0·17*	*159*	*159*	*0·14*	*39*	*39*	*0·45*	*423*	*423*	*0·45*	*108*	*108*	*0·06*	*23*	*23*	*20*
S.E.†		*81*			*17*			*316*			*116*			*13*		
Mean	2·88	2,302	2,250	0·53	354	345	4·70	2,933	2,775	4·52	733	754	0·59	91	103	154
S.E.‡		*33*			*7*			*129*			*48*			*5*		

* S.E. of differences between individual treatment means.
† S.E. of differences between method means for malonate and succinate extracts. S.E. × 2 is approximately $P<0·05$.
‡ S.E. of differences between general means for malonate and succinate extracts. S.E. × 3 is approximately $P<0·01$.
Total : % K_2O, MgO, CaO, N and P_2O_5 in dry weight.
Acetate and citrate extracts : μg. K, Mg, Ca, nitrate N and P extracted per gm. fresh weight.

than for treatments receiving nitrogen and, unlike total N determinations and the levels of nitrate N in the acetate and citrate buffers used in 1952, the malonate and succinate extracts reflected the N deficiency which was apparent in the low yields from the PK treatment.

The *phosphorus* status of the leaves was satisfactory, irrespective of the manurial treatment. There was a significant interaction between increased P status of the leaves and the omission of K.

From Table III it is seen that malonate extracts contained amounts of K, Mg, Ca, nitrate N and P similar to the succinate values.

Visual symptoms of deficiency in relation to nutrient status of leaves.

It was possible to relate the onset of deficiency symptoms in the field to the nutrient status of the leaves, as shown in Table IV.

Table IV.

Relation between the onset of visual symptoms of K, Mg, and N deficiencies and the mineral status of potato leaves.

Nutrient	Visual symptoms of deficiency produced by	Variety	24th and 25th June, 1952		18th June, 1953		Total analyses % in dry weight
			Acetate* extracts	Citrate* extracts	Malonate* extracts	Succinate* extracts	
K	trace K	Kerr's Pink (KP)	2,000	1,500	1,500	1,500	2·0
		Dunbar Standard (DS)	1,500	1,500	—	—	2·0
Mg	trace Mg	KP	800	400	300	300	0·4
		DS	900	500	—	—	0·5
NO_3	trace N	KP	—	—	500	500	—

* Values in μg. extracted per gm. fresh weight.
Acetate and citrate extracts : K, Mg and nitrate N in μg. extracted per gm. fresh weight.
Total analyses : % K_2O, MgO and N in dry weight.

Symptoms of K deficiency were observed in the *nil* and NP treatments a few days prior to the first sampling, when the K levels shown in Table IV were recorded. These threshold values for symptoms were similar for the four extractants, excepting those for Kerr's Pink determined with the acetate. Magnesium deficiency was also observed at this stage. The Mg status in the acetate extracts was higher than in either citrate, malonate or succinate buffers. The Mg values were also higher in Dunbar Standard which is not as susceptible to the deficiency as is Kerr's Pink.

The only satisfactory threshold levels for nitrate N were those determined in the malonate and succinate extracts.

DISCUSSION.

The data presented herein have already been used to show that there is a better correlation between the amounts of nutrients extracted from potato leaves by acetate or citrate buffers and the total amounts of the nutrients, than by those in malonate or succinate extracts.

In the present paper it is shown that the mean values for nutrients in the four extractants used, over all samplings, give as good an indication of nutrient status, in relation to fertilizer treatment and crop yield, as that given by the more laborious method of total analysis.

While acetate and citrate extracts are well correlated with total nutrients throughout the season and show the usual seasonal trends, viz. decrease in K and N levels and increase in Ca and Mg status, some anomalous results were obtained with malonate and succinate buffers, which seem therefore to be less suited for determining the mineral status of potato leaves.

The differences in the amounts of magnesium and calcium extracted by acetate compared with citrate may be explained on the basis of an approximately thirty-fold greater solubility of the Ca or Mg acetate as compared with the corresponding citrate. Because of such differences it is important to adopt a standard procedure when comparing the mineral status of crops.

SUMMARY.

1. The nutrient status of potato leaves, determined by total analysis and in acetate, citrate, succinate and malonate extracts, is related to manurial treatment, visual symptoms and crop yields.

2. The extraction methods, which are quick in operation, provide as good a correlation with manurial treatments, deficiency symptoms and yield as does the more tedious method of ash analysis.

3. The differential extraction of Mg and Ca by various buffers may be explained on the basis of the known solubilities of the nutrients in the extractants used.

4. Critical levels for K and Mg, coinciding with the onset of visual symptoms for these deficiencies, were determined in the four extractants and by total analysis. The K levels are similar in all extracts, except acetate, but those for Mg vary with the type of extractant used and with the potato variety.

5. Potassium deficiency restricted yields severely and the unsatisfactory K status was reflected by all methods of analysis. The nitrate N extracted in succinate and malonate buffers (1953) confirmed the limiting effect of N on the yield in the PK treatment, but the total N did not.

6. The extraction of K, Mg, Ca, P and nitrate N by the acetate buffer in the NIRD macerater is recommended as a suitable rapid method for determining the status of the nutrients in potato leaves.

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I am grateful to the following workers for their assistance : Messrs. M. A. Wright and R. T. Dewell (chemistry), Messrs. D. Rees and G. M. Clarke and Mrs. Olive James (statistical analysis) and Mr. George Jones (photography).

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BOOK REVIEWS

THE FIRST ANNUAL REPORT OF THE SCOTTISH HORTICULTURAL RESEARCH INSTITUTE 1953-1954. (Scotland: Scottish Horticultural Research Institute. 1954. Pp. 28.)

"On April 1st, 1953, the Scottish Horticultural Research Institute became legally established as a self-governing grant-aided research organization." With this opening sentence Dr. T. Swarbrick, the first Director, introduces his first Annual Report with a short historical survey of the origin and early development of this maiden research institute for Scottish horticulture.

This is heartening reading for the present writer and some of his colleagues who as long ago as the late twenties were only too well aware of the urgent research problems, both pathological and pomological, which confronted the incomparable raspberry-growing areas of Angus and Perthshire, where local stocks of well-known varieties were even then in rapid decline. Even earlier than this, the equally remarkable Lanarkshire strawberry-growing area was being shaken by the devastations of the Lanarkshire Disease, or Strawberry Red-core Root Rot as it is now better named, and by 1930 Mr. R. D. Reid (now a household name among strawberry growers) had been appointed to assist Mrs. Alcock and Dudley Howells in their studies of its cause and control. This appointment led to the breeding of the well-known Auchincruive red-core resistant varieties, beginning in 1940 and culminating in Auchincruive Climax in 1947. Meanwhile the raspberry research had also obtained a more secure *ad hoc* footing by the formation, in 1943, of the A.R.C. Scottish Raspberry Investigation, under East Malling Research Station.

It is researches on these crucial economic problems in Scottish fruit culture, together with the parallel ones posited in the well-known Scottish vegetable growing areas, notably in the Lothians (*pace* the Musselburgh Leek !), that have now been brought together and placed on a permanent institutional footing by the formation, under the authority of the Department of Agriculture for Scotland, of this new Institute.

The historical section already referred to is followed by the administrative report of the Director, with interesting details of building developments at the Mylnefield-by-Dundee headquarters and at the West of Scotland Unit (Officer-in-Charge, R. D. Reid) located at Auchincruive.

Then comes the report of the Department of Plant Pathology, under Dr. C. H. Cadman (formerly officer-in-charge of the A.R.C. Scottish Raspberry Investigation), dealing mainly with interesting recent advances in studies of virus diseases of raspberry and potato crops. The next report is devoted to the Department of Horticultural Botany under Dr. C. A. Wood, and includes sections on raspberry culture and breeding, pomological studies of strawberries, apple and plum variety-rootstock trials, the Scottish fruit variety trials and on some initial vegetable investigations.

Finally, Mr. Reid reports from Auchincruive the latest work of his Unit on strawberry and raspberry breeding and on the red-core disease.

Each report ends with an impressive first list of publications which, together

with the texts, presents an auspicious beginning to the research history of the new Scottish Institute. R.V.H.

SMALL FRUIT CULTURE. By JAMES SHELDON SHOEMAKER. (New York, Toronto, London : McGraw-Hill Book Co. 3rd Edition. 1955. Pp. 447, illus. Price $6.50 or 49s.)

Much effort in many parts of the world has been devoted to research on small fruits but the student seeking to consult the published results of all this work must have access to a very well-equipped library. Shoemaker, however, has brought together the conclusions of a large part of this research and woven them into the background of commercial practice in North America to provide a well-documented textbook. The first edition, in 1934, was in such constant demand by growers and students that reprintings appeared in 1939 and 1945. A second edition appeared in 1948 and was reprinted again in 1950. The third edition has been brought up to date and is completely changed in layout and general appearance under the production of the McGraw-Hill Book Co. The type has been re-set from beginning to end, with the use of bold type and spaces to break up the text, thus making for easier reading and the more rapid location of specific information.

The book is divided into six main chapters dealing with grapes, strawberries, bramble fruits, currants and gooseberries, blueberries and cranberries. The fullest information is given on grapes and strawberries, reflecting their greater economic importance amongst the soft fruits. Each chapter opens with the geographical distribution of the fruit in the United States and Canada and then passes on to descriptions of varieties, yields and costs, propagation, general cultural treatment, harvesting, marketing and processing. Information is also given on irrigation, herbicides, virus diseases, picking machines and the control of pests and diseases. Frequent references are given in the text to the full bibliography at the end of the book, unfortunately wrongly headed "Cranberry culture", and this list includes 621 references of which more than 100 have been published since 1950. References in the second edition to work that has since become out-dated have been omitted, thus keeping the list within reasonable limits. Little attention is paid to European publications. Numerous illustrations of good quality accompany the text and the index is well arranged and adequate.

American readers will doubtless continue to find this textbook an invaluable practical guide ; readers in other parts of the world may find it mainly useful as a source of stimulating new ideas and as background reading for the study of research literature from North America. H.B.S.M.

HORTICULTURAL SCIENCE. By GORDON T. NIGHTINGALE. (New Brunswick : Horticultural Publications, Rutgers University. 1955. Pp. 111, illus. Price $2.50.)

The title of this little book is much broader than its subject-matter. It is, in fact, intended as reading material to accompany and supplement a practical course in plant physiology for horticulture students at Rutgers University. Like so many books designed for a particular course, it is likely to be of rather limited value elsewhere. The author's name ensures that the topics of mineral nutrition and nitrogen metabolism shall be fully covered. In fact, the whole book is hung upon the peg of the carbohydrate-protein relationship ; it may be partly as a result of this that

several aspects of plant physiology, important in themselves as well as in their relevance to horticulture, are ignored completely. There is, for instance, no section dealing with water relations, respiration, translocation, growth substances or dormancy. Even enzymes and photosynthesis are barely mentioned.

This boils down, then, to a laboratory manual on plant nutrition and certain other aspects of plant physiology applied to horticulture. Even within this field, however, it is of rather limited value. The laboratory exercises described are, on the whole, elementary and would appear to have been designed for large classes which could devote little time to the subject.

Each chapter ends with a list of discussion questions and with a few references, almost all to American publications. The discussion questions are perhaps the most interesting feature of the book and many of them could form the basis for a very useful class discussion, given wise guidance. Some of them, however, smack of the leading question, and some make false assumptions. The text, too, contains a number of the didactic over-simplifications one has come to except of American textbooks at an elementary level.

The book is well printed and bound. D.W.G.

DIE MARILLE (APRIKOSE) UND IHRE KULTUR. (THE APRICOT AND ITS CULTURE.) By J. Löschnig and F. Passecker. (Vienna : Österreichischer Agrarverlag. 1954. Pp. 363, illus. Price S.(Austrian) 136 or DM. 22.30.)

This is a monograph by experts on a crop which has received all too little attention. The cover is wide, yet important aspects are dealt with in detail. Löschnig was responsible for its original conception and for many sections, Passecker for other sections and final editing after the death of Löschnig, and L. Fulmek for a short, useful section on pests and diseases.

They deal in turn with (1) general topics, such as botany and distribution of the apricot ; (2) cultural aspects, including specification of environmental needs and the determination from indicator plants whether these are met, and the valuation of trees for insurance purposes ; (3) fruit utilization, domestic and industrial ; (4) variety descriptions and (5) breeding and selection of varieties and rootstocks.

Part 4, which constitutes half the book, concerns the pomological classification and description of varieties in Europe (98), Central Asia (53), Manchuria and Siberia (6) and of the so-called cherry plum group (8) which is formed by natural hybrids between *Armeniaca vulgaris* and *Prunus cerasifera*. This section includes pioneer work by Löschnig and is well supported by colour plates and line drawings. A special feature is the description of an ingeniously simple method of getting prints from cross sections of the stone and its lateral view for identification purposes.

This publication will be of great value in all regions where the apricot is or can be grown. V.H.G.

CANE FRUITS. Ministry of Agriculture Bulletin No. 150. (London : H.M. Stationery Office. 1955. Pp. 29, illus. Price 2s. 6d.)

This bulletin, last in the series on the cultivation of soft fruits, deals with raspberries, blackberries and hybrid berries. Prepared by the Fruit Group of the National Agricultural Advisory Service, it combines necessary general information with an amount of detail surprising in a publication of such modest size. The

Ministry's Plant Pathology Laboratory, Harpenden, was responsible for the section on pests and diseases.

The new bulletin should be welcomed equally by the commercial grower seeking up-to-date information and the amateur wishing to cultivate cane fruits to best advantage. The introductory sections and those dealing with sites and soils, picking, grading and packing are especially valuable, as are also the observations on nutrition, despite the statement that few field experiments have been carried out in this country on the manurial requirements of these fruits. The information on planting, cultivation and training is practical and detailed, but reflects the need for experimental data to compare the alternative cultural methods and practices in common use. Clear descriptions, with diagrams, are given of methods of training the canes of blackberry and hybrid berries, but the timing of this work is not discussed. Although there are advantages in completing it as soon in the dormant season as possible, the severe late winter of 1954/55 produced some striking examples of the damage that may occasionally result from training these fruits to the wires too early.

Stress is rightly laid on the value of healthy planting stock and modern methods of propagation. It is satisfactory to note that, with only one or two exceptions, the raspberry varieties listed are those of which reliable stocks can fairly readily be obtained. More definite reference might have been made to the present (and, one hopes, temporary) scarcity of healthy stocks of loganberry, for the planting of this fruit is being much encouraged by the demand from the canning industry.

The final section deals with the main pests and diseases affecting cane fruits in England. Scottish readers will note the inclusion of the Raspberry Cane Midge (*Thomasiniana theobaldi* Barnes), a pest often serious in the south, and the lack of reference to the Raspberry Moth (*Lampronia rubiella* Bjerk.) and the virus disease leaf curl. Although the last, as such, has been reported only from Scotland, viruses of an apparently similar nature to that of leaf curl have lately been identified in raspberry material from southern England.

The bulletin is of handy size, clearly and logically arranged and well illustrated, and can be unreservedly recommended. C.A.W.

SCIENTIFIC HORTICULTURE, Volume XI, 1952–54. (The Horticultural Education Association, c/o Gibbs & Sons, Canterbury, Kent. 1955. Pp. 198, illus. Price 14s.)

This eleventh volume is as interesting as its predecessors, describing in non-technical language the latest trends in scientific thought and practice in regard to horticultural matters.

E. W. Russell gives a veiled warning as to reliance on rotary cultivation to the exclusion of the plough, and this should be taken seriously by heavy-land farmers.

There is much information on cherries. N. B. Bagenal writes on the history of cherry growing. N. H. Grubb and M. B. Crane also write on cherries. The latter gives an up-to-date compatibility table including the Merton varieties. Garner, on cherry tree production, mentions the "staddle" method of budding which has in fact been quite successful in practice in reducing losses by bacterial canker.

Among the most informative articles are those on plant viruses. Methods of testing and diagnosis are dealt with, as are the more fundamental principles involved in the study of viruses.

L. P. Smith writes on irrigational requirements from the meteorological aspect ;

his information might be studied with reward by those contemplating irrigation on a commercial scale.

Much ground is covered on the breeding of brussels sprouts, winter cauliflower and tomatoes, and there is some information of interest to glasshouse growers.

There is less on nutritional subjects than one might expect, and entomology is chiefly confined to eelworm.

There are six pages of illustrations. A.H.

PUBLICATIONS RECEIVED.

ENIGE ERVARINGEN MET HET VEREDELEN VAN BONEN (1). Edited by Tj. BUISHAND. Proefstation voor de Groenteteelt in de Volle Grond in Nederland. No. 1 (July, 1955).

ANNUAL REPORT OF THE BOTANIC GARDENS DEPARTMENT FOR 1954, COLONY OF SINGAPORE. By J. W. PURSEGLOVE. (Singapore : Government Printing Office. 1955. Pp. 11, illus. Price 50c.)

AMATEUR GARDENING DIARY FOR 1956. (London : W. H. & L. Collingridge Ltd. 1955. Price 7s.)

LANDELIJKE BEPROEVING VAN ENIGE STAMSLABONENRASSEN. Ir. W. A. WIEBOSCH and Tj. BUISHAND. Proefstation voor de Groenteteelt in de Volle Grond in Nederland. No. 2 (September, 1955).

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The Publication Committee welcome horticultural papers from all other sources.

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4. Each paper must conclude with a concise and critically correct **summary** of its actual content. It should contain any new or verified constants as well as any other critical data of permanent value but not tables or graphs. Any organism mentioned should be given its scientific name.

5. Where a paper is of a highly technical nature it should be preceded by a brief **synopsis**, in which its contents are simply and clearly stated for the benefit of non-technical readers.

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